Real-time free cortisol quantification among critically ill children

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Objectives: **Ascertainment of adrenal function assessing free rather that total cortisol may be beneficial for the diagnosis of critical illness-related cortisol insufficiency. We hypothesized that centrifugal ultrafiltration would provide timely free cortisol data that highly correlated with the gold standard, but logistically cumbersome, equilibrium dialysis technique when the free cortisol fractions were identically quantified by chemiluminescence immunoassay. We also hypothesized that free cortisol would correlate with illness severity in a large cohort of critically ill children.**

Design: **Prospective, multi-institutional, observational cohort investigation.**

Setting: **Seven pediatric intensive care units within the *Eunice Kennedy Shriver* National Institute of Child Health and Human Development Collaborative Pediatric Critical Care Research Network.**

Patients: **One hundred sixty-five critically ill children across the spectrum of illness severity.**

Interventions: **Blood sampling.**

Measurements and Main Results: **Time to derive plasma free cortisol concentrations after centrifugal ultrafiltration or equilibrium dialysis fractionation with chemiluminescence immunoassay was approximately 2 vs. approximately 24 hrs, respectively. Using centrifugal ultrafiltration, mean plasma free cortisol was 4.1 ± 6.7 μg/dL (median, 1.6 μg/dL; range, 0.2–43.6 μg/L), representing an average of 15.2 ± 9.4% of total cortisol. Nearly 60% of subjects exhibited free cortisol <2 and 30% <0.8 μg/dL, previously suggested threshold concentrations for defining critical illness-related cortisol insufficiency. Plasma-free cortisol concentrations comparing centrifugal ultrafiltration vs. equilibrium dialysis fractionation demonstrated a strong correlation (R^2 = 0.97). For free cortisol <2 μg/dL, Bland-Altman analysis revealed minimal negative bias for the centrifugal ultrafiltration technique. Illness severity assessed by Pediatric Risk of Mortality III correlated moderately with free cortisol and percent total cortisol as free cortisol.**

Conclusions: **Determination of centrifugal ultrafiltration fractionated free cortisol was fast and results correlated highly with equilibrium dialysis fractionated free cortisol. Many children exhibited free cortisol <2 and <0.8 μg/dL but did not demonstrate clinical evidence of critical illness-related cortisol insufficiency. This study ascertains that real-time free cortisol quantification is feasible to potentially help guide clinical decisionmaking for cortisol replacement therapy in the pediatric intensive care unit. (Pediatr Crit Care Med 2011; 12:000–000)**

KEY WORDS: cortisol; total cortisol; free cortisol; hydrocortisone; children; equilibrium dialysis; centrifugal ultrafiltration; radioimmunoassay; chemiluminescence immunoassay; adrenal insufficiency; relative adrenal insufficiency; critical illness; critical illness related cortisol insufficiency (CIRCI)

Stress from critical illness activates a neurogenic–endocrine–inflammatory response, including activation of the hypothalamic–pituitary–adrenal axis resulting in increased cortisol production (1, 2). Cortisol plays a key role in the compensatory anti-inflammatory response syndrome in the setting of an evolving systemic inflammatory response syndrome. Critical illness-related cortisol insufficiency (CIRCI) defines a seemingly inadequate hypothalamic–pituitary–adrenal–cortisol response relative to the intensity of stress. This may involve insufficient *de novo* adrenal production of cortisol, altered cortisol transport, or peripheral tissue resistance to cortisol (3–

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5). Defining appropriate adrenocortical status in the setting of critical illness remains crucial but confusing (6). Despite significant clinical research in this area, there is no consensus on what constitutes an adequate adrenal response to severe stress such as septic shock (7). Historically adrenal function has been assessed with serum total cortisol (TC) concentrations typically at baseline and/or after adrenal stimulation with exogenous corticotropin. The Corticosteroid Therapy of Septic Shock (CORTICUS) interventional trial examined the potential benefits and risks of adjunctive cortisol for adult septic shock. An important conclusion of this trial was that corticotropin adrenal stimulation testing that measures serum TC did not reliably identify a subgroup of critically ill septic adults who might benefit from exogenous cortisol replacement (8).

An evolving consensus suggests that free cortisol (FC) rather than protein-bound cortisol is responsible for the protean actions of this hormone (9, 10). Normally cortisol binding globulin (transcortin) and albumin bind >90% of circulating cortisol. During critical illness, the concentrations of these proteins may plummet by ≥50%, but individual variation is significant. Among critically ill adults with sepsis and septic shock, FC concentrations may correspond more closely to illness severity than TC (9, 11).

For the measurement of FC, two methodologic considerations are important: 1) FC fractionation from protein-bound cortisol; and 2) actual cortisol assay. Traditionally isolation of the FC fraction has been accomplished using equilibrium dialysis (EQD). This technique is typically a "send out" test for most clinical laboratories, requires relatively large blood volumes, and involves prolonged incubation times to generate the FC fraction. More recently, temperature-controlled centrifugal ultrafiltration (CUF) (12) was suggested as an alternative method for FC fractionation. This approach requires only small blood volumes and has a short turnaround time. These test characteristics would make real-time FC measurements feasible among critically ill children.

While designing an interventional trial of adjunctive cortisol replacement for children with severe sepsis/septic shock, the Collaborative Pediatric Critical Care Research Network investigators realized the importance of identifying the population of critically ill children who would most likely benefit from such an intervention. We sought to generate TC and FC data on a cohort of critically ill children across a spectrum of ages and illness severity. We hypothesized that plasma FC fractionated by temperature-controlled CUF is equivalent to plasma FC fractionated by the gold standard EQD technique when a common assay method (chemiluminescence immunoassay) is used. We further hypothesized that FC would correlate with severity of illness.

MATERIALS AND METHODS

Institutional Review Board. This project was reviewed and approved by the Institutional Review Boards of Seattle Children's Hospital, each Collaborative Pediatric Critical Care Research Network clinical performance site, and the Collaborative Pediatric Critical Care Research Network Data Coordinating Center.

Cortisol Quantification Investigation Protocol. Within Collaborative Pediatric Critical Care Research Network pediatric intensive care units (PICUs), critically ill children with a wide spectrum of illness severity were screened for enrollment by Collaborative Pediatric Critical Care Research Network research coordinators and investigators during their first day after admission to PICUs. Enrolled subjects met all inclusion criteria and no exclusion criteria.

Inclusion criteria included: admission to a PICU; age >40 wks gestation and <18 yrs; weight >5 kg; blood sample obtainable within 24 hrs of PICU admission; and an indwelling vascular catheter capable of providing blood samples.

Exclusion criteria included: administration of systemic steroid within the previous month; lack of commitment to aggressive intensive care therapy (children with limited resuscitation orders); subject *status post* cardiac surgery requiring cardiopulmonary bypass, extracorporeal membrane oxygenation, leukapheresis, plasmapheresis, ultrafiltration, any dialysis therapy or massive transfusion (>50% of total blood volume); subject was not expected to survive PICU admission; subject was previously enrolled in the same study; or subject was administered etomidate or ketoconazole within the previous month.

Research Procedures. After screening identification of appropriate cortisol quantification investigation subjects, parental permission was obtained for data collection and a single blood sample from an indwelling vascular catheter. Demographic data were recorded. Illness severity was assessed for each child using the Pediatric Risk of Mortality version III score (PRISM III) using data collected during the first 12 hrs of PICU admission (13). Each subject was also assessed for the systemic inflammatory response syndrome (systemic inflammatory response syndrome, presence of tachypnea, tachycardia/bradycardia, hyper-/hypothermia, and leukocytosis/leukopenia with a left shift on differential) using published consensus criteria (14). A single 4-mL blood sample was drawn from each subject using existing intravascular access. Plasma samples were isolated by centrifugation immediately after blood collection, frozen, and subsequently shipped on dry ice to Seattle Children's Hospital. Integrity of each frozen sample at arrival to Seattle was ascertained. Each sample underwent concurrent fractionation by EQD and CUF. For comparison, existing plasma samples from 21 healthy, unstressed adults were identically processed and assayed.

Free Cortisol Fractionation. Temperature-controlled CUF was conducted according to the method of Lentjes et al (12) using Millipore YM-30 membrane filters (molecular weight cutoff 30,000) (Millipore, Billerica, MA). Plasma samples (0.40 mL) were centrifuged at 1700 × *g* for 30 mins at 37°C in a prewarmed centrifuge to yield a protein-poor FC fraction. Equilibrium dialysis was performed using the Rapid Equilibrium Dialysis Device, Pierce Protein Research Products (Thermo Fisher Scientific Inc, Rockford, IL).

Cortisol Assay. Both TC and FC (after CUF or EQD) were measured at the Clinical Laboratory of Seattle Children's Hospital using the Ortho Clinical Diagnostics ECi Immunodiagnostic System using enhanced chemiluminescence detection. Total assay time on the analyzer was 38 mins.

Data and Statistical Analyses. Conversion of cortisol units was nM × 0.0362 = μg/dL; μg/dL × 27.6 = nM. PRISM III scores were categorized as follows to reflect different levels of illness severity: 0–7, 8–15, 16–23, and ≥24. Continuous variable data were summarized using the mean ± 1 SD, median, and 25th and 75th percentiles. Dichotomous variables were summarized as percentages. We performed linear regression to examine correlation between the different measures. Agreement between EQD FC and CUF FC was also evaluated using the Bland-Altman approach (15, 16) for all FC values and for FC values restricted to <2 μg/dL.

RESULTS

Study Enrollment. Parents provided permission for enrollment for 174 of 201 eligible subjects (87%). Blood samples were obtained from 165 enrolled subjects (95%) within the first 24 hrs of PICU admission in all but one subject with an average time from admission to blood sampling of 16.1 ± 4.9 hrs (range, 1.6–25.1 hrs). One atypical outlier observation was overly influential in the results as a result of TC and FC values of 281 and 150 μg/dL, respectively. These values were excluded from all analyses.

Table 1. Demographics of the study population

Characteristic	Number	Percent	Mean	SD	Median	Q1, Q3
Age	165	—	9.7	5.7	11.3	3.9, 14.7
PRISM III score	165	—	8.0	6.9	8.0	3.0, 11.0
PRISM III score category						
0–7	82	49.7				
8–15	63	38.2				
16–23	15	9.1				
≥24	5	3.0				
Gender						
Male	86	52.1				
Female	79	47.9				
SIRS diagnosis						
Yes	127	77.0				
No	38	23.0				
Chronic diagnoses						
Yes	88	53.3				
No	77	46.7				

Q1 and Q3, 25th and 75th percentiles; PRISM, Pediatric Risk of Mortality Score, version III; SIRS, systemic inflammatory response syndrome.

Table 2. Acute primary PICU admission diagnoses for the study population

Diagnosis	Number	Percent
Sepsis/meningitis/pneumonia	26	15.1
DKA, other metabolic disease	22	12.8
Trauma	20	11.6
Postoperative orthopedic surgery	20	11.6
Postoperative neurosurgery	13	7.6
Postoperative CHD surgery	8	4.7
Seizures, other neurological	8	4.7
Hypoxia–ischemia–reperfusion	7	4.1
Cancer	7	4.1
Acute abdomen	6	3.5
Acquired cardiovascular disease	5	2.9
Renal failure	5	2.9
Hematologic disease	5	2.9
Hepatic failure	4	2.3
Other diagnoses	16	9.3

PICU, pediatric intensive care unit; DKA, diabetic ketoacidosis; CHD, congenital heart disease.

Table 3. Chronic primary diagnoses for the study population

Diagnosis	Number	Percent
Motor/mental developmental delay	34	26.2
Diabetes, other metabolic disease	17	13.1
Cardiovascular disease	10	7.7
Cancer	9	6.9
Other chronic neurological disease	9	6.9
Hydrocephalus, MMC, Chiari	8	6.2
Chromosomal abnormalities	8	6.2
Chronic seizure disorder	7	3.4
ADHD, autism	5	3.8
Chronic gastrointestinal disease	5	3.8
Defined syndromes	5	3.8
Chronic orthopedic disease	5	3.1
Bronchopulmonary dysplasia	3	2.3
Asthma	3	2.3
Psychosocial disorders	3	2.3

MMC, meningomyelocele; Chiari, Chiari malformation; ADHD, attention deficit hyperactivity disorder.

Specific diagnoses were combined to yield MMC, Chiari, and ADHD. Categories are not mutually exclusive; subjects may be included in more than one category.

Characteristics of the Study Population. Demographics for the study population are summarized in Table 1, whereas primary PICU admission diagnoses are summarized in Table 2 and the most commonly encountered chronic diagnoses summarized in Table 3.

Healthy Adult Controls. Table 4 summarizes TC, FC, and FC as a percent of TC using the CUF and chemiluminescence immunoassay for 21 healthy, unstressed adults. Morning TC averaged approximately 10 μg/dL and FC approximately 1 μg/dL with FC comprising approximately 10% of TC. Afternoon TC and FC concentrations were approximately 60% of morning values with FC again comprising approximately 10% of TC.

Critically Ill Children. Table 5 summarizes TC, FC, and FC as a percent of TC derived using both EQD and CUF plasma fractionation methodologies and chemiluminescence immunoassay for the 164 critically ill children.

These PICU patients exhibited roughly a 100% higher TC and a 100–400% higher FC compared with the adult control subjects. FC as a percent of TC in this pediatric population was approximately 60% greater than adult controls (ie, approximately 16% in the critically ill children vs. approximately 10% in the healthy adults). However, for a few children, the FC fraction comprised 50–60% of the TC.

EQD Versus CUF FC Fractionation. Direct comparison based on linear regression as well as Bland-Altman analysis of the relationship between EQD and CUF plasma fractionated FC is displayed in Figure 1. Among the 94 children with free cortisol <2 μg/dL by either fractionation technique (in which providers might be considering cortisol replacement therapy), a slight negative bias for the CUF compared with the EQD fractionation technique was apparent. The mean bias (EQD FC − CUF FC) was 0.20 ± 0.31 μg/dL with lower and upper limits of agreement of −0.41 and 0.81, respectively. Process time for CUF vs. EQD fractionation/analysis was approximately 2 vs. 24 hrs. This difference was the result of the prolonged time needed for dialysis vs. ultracentrifugation to isolate the FC fraction.

As displayed in Figure 2, the relationship between FC and TC was quadratic. These graphs demonstrate the nonlinear binding characteristics between cortisol and its binding proteins (17).

The relationship of FC as expressed as a percentage of TC is displayed in Figure 3. In general, as TC increases, the percent of cortisol as the FC fraction increases, but there is considerable variation, particularly at low TC and FC concentrations.

Free Cortisol Relation to Illness Severity. For this cohort, 33% of children exhibited TC ≤10; 57% exhibited FC <2 μg/dL by either EQD or CUF and 30% exhibited FC <0.8 μg/dL by either EQD or CUF. These reference TC and FC concentrations were previously suggested as potential thresholds for defining adrenal insufficiency in critically ill adults based on direct TC and FC assessment and metyrapone testing (9, 18). No subjects in any of these subgroups developed clinical symptoms suggestive of adrenal insufficiency such as recalcitrant shock, severe hyponatremia, or hypoglycemia. Seventy-seven percent of subjects (126 of 164) fulfilled criteria for systemic inflammatory response syndrome. As

Table 4. Total cortisol, free cortisol, and free cortisol as percent of total cortisol concentrations among 21 healthy, nonstressed adults assessed with paired serum samples obtained at approximately 8:00 AM and 4:00 PM using temperature-controlled centrifugal ultrafiltration and chemiluminescence immunoassay methodology[a]

	8:00 AM Blood Sample			4:00 PM Blood Sample		
Measure	Total Cortisol	Free Cortisol	Free as Percent of Total	Total Cortisol	Free Cortisol	Free as Percent of Total
Mean ± SD	10.5 ± 5.1	1.0 ± 0.4	9.9 ± 2.6	6.6 ± 2.7	0.6 ± 0.2	9.6 ± 2.7
Median	9.0	0.9	10.0	6.0	0.6	9.7
Minimum, maximum	5.0, 27.8	0.6, 2.1	4.6, 15.0	2.3, 9.9	0.3, 1.1	4.5, 16.7

[a]All concentrations are expressed as μg/dL. n = 21.

Table 5. Total cortisol, free cortisol, and free cortisol as a percent of total cortisol fractionated by two methods[a]

		Free Cortisol Fractionation Method			
		Equilibrium Dialysis (EQD)		Centrifugal Ultrafiltration (CUF)	
Measure	Total Cortisol	Free Cortisol	Free as % of Total Cortisol	Free Cortisol	Free as % of Total Cortisol
Mean ± SD	20.1 ± 18.3	3.8 ± 5.7	16.6 ± 9.9	4.1 ± 6.7	15.2 ± 9.4
Median	14.3	1.9	14.1	1.6	12.8
Minimum, maximum	0.6, 109	0.2, 37.2	4.5, 67.9	0.2, 43.6	3.6, 48.3

[a]All concentrations expressed as μg/dL. n = 164.

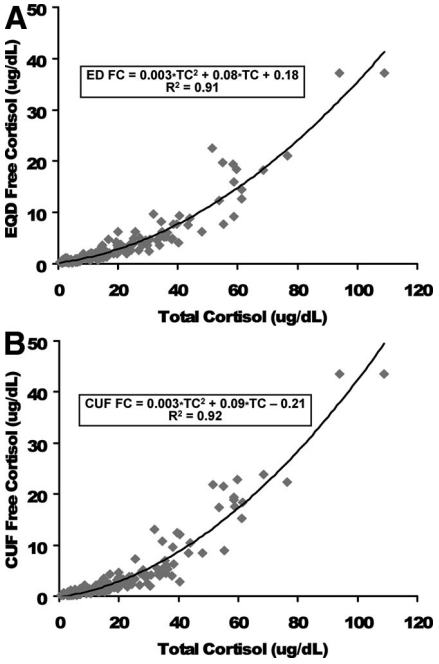

Figure 2. Free cortisol as a function of total cortisol after plasma fractionation per equilibrium dialysis (left figure, *EQD*) or temperature-controlled centrifugal ultrafiltration (right figure, *CUF*). Cortisol concentrations are expressed as μg/dL. *TC*, total cortisol; *FC*, free cortisol.

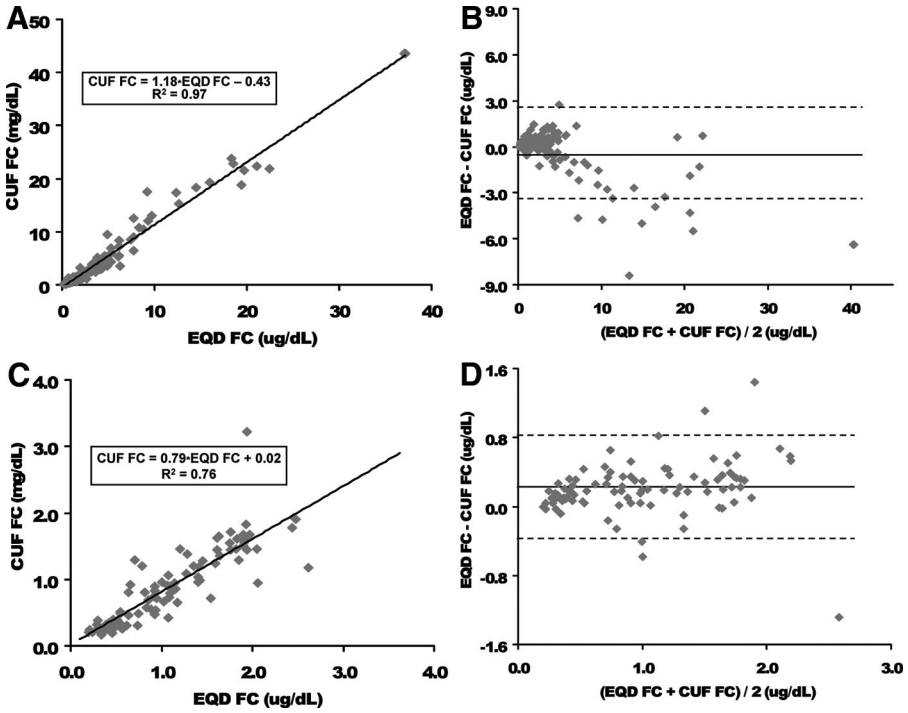

Figure 1. Free cortisol per centrifugal ultrafiltration (*CUF*) plasma fractionation as a function of free cortisol per equilibrium dialysis (*EQD*) plasma fractionation. A comparison of the CUF vs. EQD techniques is displayed for the entire population (n = 164) in the upper graphs and for the population of children with a free cortisol level <2 μg/dL (n = 94) in the lower graphs. Cortisol concentrations in μg/dL. *CUF FC*, centrifugal ultrafiltration free cortisol; *EQD FC*, equilibrium dialysis free cortisol.

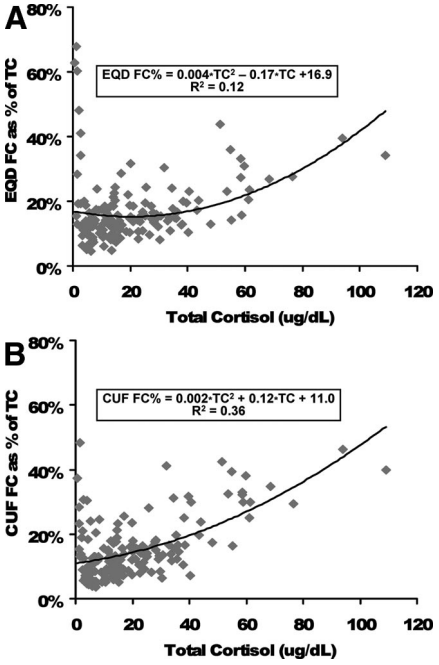

Figure 3. Percent of total cortisol as free cortisol after plasma fractionation per equilibrium dialysis (left figure, *EQD*) or temperature-controlled centrifugal ultrafiltration (right figure, *CUF*). Cortisol concentrations are expressed as μg/dL. *TC*, total cortisol; *FC*, free cortisol.

Table 6. Total cortisol and free cortisol fractionated by equilibrium dialysis (EQD) or centrifugal ultrafiltration (CUF) among subjects without (SIRS−, n = 38) or with (SIRS+, n = 126) systemic inflammatory response syndrome (SIRS)

	Total Cortisol		EQD Free Cortisol		CUF Free Cortisol	
Measure	SIRS−	SIRS+	SIRS−	SIRS+	SIRS−	SIRS+
Mean ± SD	18.8 ± 16.1	20.5 ± 19.0	3.1 ± 3.5	4.0 ± 6.2	3.1 ± 4.4	4.3 ± 7.3
Median	14.2	14.3	1.9	1.9	1.6	1.6
Minimum, maximum	1.4, 61.2	0.6, 109	0.3, 16	0.2, 37.2	0.2, 19.3	0.2, 43.6

Table 7. Total and free cortisol values in relation to PRISM III categories

PRISM III Score Category	Median TC	Median CUF FC	Median FC as Percent TC
0–7	11.7	1.3	12.4
8–15	19.3	2.3	12.3
16–23	17.0	2.3	15.5
≥24	53.7	17.4	32.4

PRISM, Pediatric Risk of Mortality; TC, total cortisol; CUF, centrifugal ultrafiltration; FC, free cortisol per centrifugal ultrafiltration fractionation.

summarized in Table 6, and in contrast to our expectations, no statistically significant differences occurred in TC and FC concentrations among subjects with and without systemic inflammatory response syndrome.

Pearson correlation coefficients for PRISM score vs. cortisol concentrations were: TC, 0.36; EQD FC, 0.33; and CUF FC, 0.38. Pearson correlation coefficients for PRISM score vs. EQD FC as percent TC and CUF FC as percent TC were 0.12 and 0.34, respectively. As shown in Table 6, median TC, CUF FC, and CUF FC as percent TC values increased with increasing PRISM III categories. Whereas TC increased 4.6-fold over the PRISM illness severity categories, CUF FC increased 13.4-fold. Free cortisol as percent TC increased 2.6-fold over the PRISM illness severity categories.

DISCUSSION

In this study, we have demonstrated that plasma cortisol may be fractionated using clinically relevant, real-time, temperature-controlled CUF rather than a prolonged EQD technique preparatory to measurement of FC by chemiluminescence immunoassay. Both TC and FC concentrations as well as FC as a percent of TC were markedly higher among critically ill children compared with unstressed adult volunteers. Illness severity per PRISM III correlated moderately with TC, FC, and FC as percent TC. Although nearly 60% of the children exhibited FC <2 and 30% <0.8 μg/dL, none were suspected of clinical CIRCI. These data suggest that FC measurement is feasible in real time and should be further investigated among specific populations of critically ill children with outcome data to potentially clarify the diagnosis of pediatric CIRCI. Our data also suggest that FC <2 or perhaps even <0.8 μg/dL is probably not an appropriate definition for CIRCI in children.

Most circulating cortisol is protein-bound to albumin and transcortin. However, the FC fraction is responsible for the biologic effects of the hormone. In general, measurement of total serum/plasma concentrations of thyroid and steroid hormones has limited biologic validity, because the bioavailability of these hormones is substantially determined by variable concentrations and binding characteristics of their respective hormone-binding proteins (19). It is recognized that concentrations of the cortisol-binding proteins may change considerably with critical illness.

Variously derived cortisol concentrations have been suggested in an attempt to identify CIRCI using a random baseline TC or incremental increase in TC after high- or low-dose corticotropin (6). However, a key conclusion of the largest trial examining adjunctive hydrocortisone for adult septic shock, CORTICUS, was that that the short, high-dose corticotropin adrenal stimulation test, as it is currently being used, did not appear to be useful for identifying a population with adrenal insufficiency who would most likely benefit from hydrocortisone supplementation (8). Accordingly, it has been suggested that FC rather than TC may be the preferred measure of adrenal sufficiency particularly for critically ill patients (9, 11). It is acknowledged that a recent prospective multicenter investigation of adrenal function among 381 critically ill children using low-dose corticotropin adrenal stimulation reported a significant increase requirement for fluid boluses and vasoactive–inotropic infusions among children with stimulated minus baseline TC <9 μg/dL (20).

Equilibrium dialysis or ultrafiltration/ligand binding methods have traditionally been used to isolate FC, but these methodologies have been hampered by expense, technical difficulties, large-volume serum requirement, long analysis times, or need for a radionuclide tracer (21). Equilibrium dialysis additionally requires correction for the dilution-induced shift of binding equilibria. Furthermore, for many clinical laboratories, FC assessment by EQD or liquid chromatography/mass spectroscopy (22) represents a "send out" study that requires days for reporting results. On the other hand, CUF with chemiluminescence immunoassay requires approximately 2 hrs from receipt of blood specimen to reporting results.

Results reported here comparing healthy adult control subjects with critically ill children are very similar to those previously reported comparing healthy adult control subjects with a general population of critically ill adults using EQD fractionation and immunoradiometric assay (9); comparing healthy adult control subjects with stressed medical and surgical patients using EQD fractionation and chemiluminescence immunoassay (23); and comparing healthy adult control subjects with septic adults using a ligand binding/ultrafiltration methodology (11).

More recently FC was measured directly after temperature-controlled CUF of serum/plasma samples (12, 19). This alternative approach avoids radioactivity, is faster, and accommodates smaller serum/plasma volumes. A recent comparison of CUF and EQD fractionation methodologies concluded that both produce acceptable reproducibility with very similar results when the filtrate or dialysate is assessed by the same automated immunoanalyzer system (19). Using CUF methodology essentially identical to that used in the current study, investigators reported CUF FC concentrations for 115 healthy adults with age ranging from 18 to 60 yrs with a median value of 0.86 μg/dL (23.8 nM [range, 0.43–1.56 μg/dL; 12–43 nM]) representing 4.0–9.5% of TC (12).

Results reported here for FC values among critically ill children are similar to those reported for critically ill adults (also using CUF with a YM-30 filter with 30-kDa cutoff for serum fractionation and an immunoanalyzer) (19). In that investigation, FC for the critically ill adults averaged 6.55 μg/dL (181 nM) representing 25.0% of TC as compared with healthy control subjects with FC 0.63 μg/dL (17.4 nM) representing 4.0% of TC. In the same investigation, EQD was compared with CUF and demonstrated a slight positive bias with EQD FC = 1.2 * CUF FC + 3.9 (nM).

FC may also be measured directly from saliva samples, eliminating the need for serum or plasma fractionation (24). Salivary cortisol is in equilibrium and correlates with the free (unbound) fraction of the hormone in the circulation. Gender-specific morning salivary cortisol reference values have been published (25). However, reduced flow of saliva may be encountered among critically ill patients (26). Furthermore, any oropharyngeal blood (instrumentation from suctioning, tracheal intubation) will contaminate a salivary sample with blood-derived cortisol.

FC may also be calculated knowing the concentrations of TC and the cortisol-binding proteins, albumin, and cortisol-binding protein (transcortin) (27). For example, den Brinker et al (28) calculated "bioavailable cortisol" in children with meningococcal sepsis or septic shock. Unlike previous adult studies, these investigators found no difference in cortisol-binding protein and albumin concentrations between control subjects and adults surmising that this reflected considerable use of plasma proteins for volume resuscitation. This may reflect why calculated "bioavailable cortisol" concentrations were not more informative than TC in informing adrenal sufficiency. Similarly, Bendel et al (29) compared TC and calculated FC in septic adults using the Coolens' method and again discerned no difference in terms of predicting outcomes. Although this study demonstrated good correlation between TC and calculated FC, the authors noted that other investigators have concluded that despite good correlation of measured and calculated serum FC, there may be a significant mean percentage difference between both methods and significant individual differences (19). These investigators further pointed out that they used Coolens' calculated FC instead of direct FC measurement because of the time-consuming, expensive equilibrium dialysis methodology, precisely the focus on the current investigation.

In fact, the concentrations of both of the cortisol-binding proteins may vary considerably, particularly among critically ill patients (9, 30). Other investigators have concluded that FC calculation using the Coolens' equation significantly underestimates FC as compared with direct measurements (19). Mathematically calculating FC using three independent variables (TC, cortisol binding globulin, albumin), each with inherent variability, seems inferior to directly quantifying FC with a real-time, reasonable expense methodology.

Potential advantages of the CUF method include smaller plasma volumes (approximately 0.40 mL) as well as a rapid turnaround time (approximately 2 hrs) with chemiluminescence immunoanalyzers commonly available in most clinical laboratories. In this investigation, the cassette EQD methodology used uses smaller plasma volumes than traditional EQD but is not widely available in clinical laboratories and still would not provide data for real-time clinical decisionmaking for critically ill patients (31).

An important limitation of the current study relates to the fact that TC and FC concentrations were not analyzed with respect to patient outcomes. Although effort was made to enroll children across the entire spectrum of illness severity, children with lower PRISM scores are more heavily represented. When standard, accepted methodologies emerge for both FC fractionation and FC analysis, FC concentration ranges among normal children of various ages will also need to be determined, because TC varies not only with illness severity, but also with age (32). However, given that nearly 60% and 30% of the current cohort exhibited FC <2 and <0.8 μg/dL, respectively, without evidence of clinical manifestations of CIRCI, this investigation calls into question the use of such thresholds for assigning a diagnosis of CIRCI among critically ill children.

With respect to cortisol kinetics in pediatric sepsis, the next logical investigation should be designed to quantify both TC and FC concentrations at presentation among children with a spectrum of sepsis illness severity. Long-term follow-up of clinically meaningful outcome measures should be included with an eventual goal of identifying the population with the highest benefit/risk ratio of pharmacologic corticosteroid intervention for CIRCI, however it may be defined. Use of the CUF methodology for FC analysis appears to be a valid, usable tool to facilitate such studies. Current data suggest that real-time FC quantification is possible to ultimately facilitate clinical decisionmaking regarding cortisol replacement therapy for children with critical illness.

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