Longitudinal Assessment of Adrenal Function in the Early and Prolonged Phases of Critical Illness in Septic Patients: Relations to Cytokine Levels and Outcome

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Context: Adrenal dysfunction remains a controversial issue in critical care. The long-stay intensive care unit (ICU) population may be at increased risk of adrenal insufficiency.

Objective: We aimed to determine whether adrenal dysfunction develops during the course of sepsis.

Design: This is a prospective observational longitudinal study.

Setting: The study was conducted in the ICU of a secondary/tertiary care hospital

Patients: We studied 51 consecutive mechanically ventilated patients with sepsis.

Intervention: We measured cortisol, ACTH, cortisol-binding globulin, cytokines, and cortisol 30 minutes after 1 μg ACTH(1–24), upon sepsis diagnosis and every 3 to 4 days, until Day 30 or until recovery or death.

Main Outcome Measures: We looked for changes in baseline and stimulated cortisol levels and its relationship to ACTH levels, sepsis severity or survival.

Results: Baseline and stimulated cortisol levels did not vary significantly. Septic patients with shock had higher baseline (20 ± 6 vs 17 ± 5 μg/dL, $P = .03$) and stimulated cortisol levels (26 ± 5 vs 23 ± 6 μg/dL, $P = .04$), compared with those without shock. On Day 1, ACTH levels could not predict cortisol levels ($R^2 = 0.06$, $P = .08$). ACTH levels increased significantly after Day 10 and, at this time point, they related to cortisol levels ($R^2 = 0.35$, $P < .001$). Development of septic shock, or resolution from it, was not associated with changes in baseline, stimulated cortisol levels, or the cortisol increment. There was much inpatient variability in the diagnosis of adrenal dysfunction at different time points.

Conclusions: Total cortisol levels relate both to the severity and outcome of sepsis and remain fairly unchanged during the course of illness. Initially, cortisol levels are largely ACTH independent, whereas ACTH increases and correlates with cortisol levels later on. Adrenal dysfunction does not seem to be a major problem during the prolonged phase of sepsis. Although not significant, the variation in cortisol levels may be such that classification of patients varies, questioning the utility of arbitrary cut-offs to define adrenal dysfunction in septic patients. (***J Clin Endocrinol Metab* 99: 4471–4480, 2014**)

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Abbreviations: APACHE, Acute Physiology and Chronic Health Evaluation; CBG, cortisol-binding globulin; ΔF, cortisol increment; FCI, free cortisol index; ICU, intensive care unit; NS, nonsignificant; ROC, Receiver Operating Characteristic; SOFA, Sequential Organ Failure Assessment.

During the past decades, impaired adrenal function has been implicated in the sepsis-related poor outcome, especially in patients with septic shock (1–3). Terms such as critical illness–related corticosteroid insufficiency and relative adrenal insufficiency were introduced to describe inadequate cortisol responses to the magnitude of stress. Currently, this entity represents one of the most controversial issues in critical care (4). Because the clinical setting is not specific, the applied biochemical criteria are arbitrary, variable, questionable, and interventional studies failed to demonstrate any benefit from glucocorticoid administration (5–7).

One confounding factor is that most studies assessed patients' adrenal function at a single time point that varied greatly from within 24 hours to up to 2 months after the onset of sepsis (4). It is accepted that the acute (spanning the first days) and prolonged (from 7 to 10 days onward) phases of critical illness exhibit different neuroendocrine alterations (8). It has recently been shown that ACTH levels are unexpectedly low during the acute phase of critical illness (9), causing potentially adrenal atrophy later on. Accordingly, it has been suggested that it is the long-stay intensive care unit (ICU) population that may be at increased risk of adrenal dysfunction. So far, cortisol (4, 9–13), and less frequently, ACTH (9, 12) levels were assessed for up to 10 days in a small number of studies, whereas there are no data on repeated low-dose (1 μg) synthetic ACTH 1–24 testing, a sensitive test to detect adrenal exhaustion and/or atrophy.

Herein we prospectively investigated a cohort of mechanically ventilated septic patients longitudinally, over a 1-month period. We looked for any changes in the adrenal secretory activity, as assessed by unstimulated and 1-μg ACTH 1–24-stimulated cortisol levels and its relationship to basal ACTH levels. Our aim was to determine a crucial time, if any, in which there is biochemical evidence of impaired adrenal function with a significant affect on clinical correlates such as development of septic shock or survival. We focused on the development of septic shock due to the clinical similarities of this condition with adrenal insufficiency. Becasue cytokines are known to have an effect on the hypothalamic-pituitary-adrenal axis (14), a number of cytokines were also serially determined.

Materials and Methods

Study population

This is a prospective study that was conducted in a 30-bed general ICU during a 1-year period. Institutional Review Board approved the study and informed consent was obtained from patients' relatives. Mechanically ventilated consecutive patients were included within 24 hours of diagnosis of sepsis, severe sepsis, or septic shock according to the American College of Chest Physicians/Society of Critical Care Medicine consensus statement (15). Exclusion criteria were: less than 18 years of age, HIV infection, and administration of drugs afflicting the hypothalamic-pituitary-adrenal axis prior or during ICU stay. In six patients, glucocorticoids were initiated during the study period, based on the treating physician's choice and were analyzed until glucocorticoid administration, but excluded from any mortality analysis. Details and a separate analysis of these patients are presented as Supplemental Results.

Twelve patients developed septic shock during the study protocol, one of them twice; eight after day 1 (two on day 4, two on day 7, three on day 10, one on day 22), whereas four presented with septic shock that subsequently resolved and reappeared (one on day 10, three on day 18).

Endocrine evaluation and cytokine measurements

We performed initial blood sampling on the day of sepsis diagnosis (herein day 1) and thereafter on days 4, 7, 10, 14, 18, 22, 26, and 30 or upon termination of mechanical ventilation or death. Blood was drawn between 0800 h and 0830 h to measure cortisol, ACTH, cortisol-binding globulin (CBG), cytokines IL-1β, IL-2, IL-6, IL-8, IL-10, IL-12p70, and TNF-α. Samples for ACTH measurements were kept in ice, spun at 4°C, and stored at −20°C until assayed. The free cortisol index (FCI) was calculated as FCI = [total cortisol (nmol/L)/CBG (mg/L)] (10). To convert cortisol values from μg/dL to nmol/l we multiplied by 27.6. After blood sampling we administered IV 1 μg synthetic ACTH 1–24 (tetracosactrin acetate; Synacthen) and measured cortisol at 30 minutes. We calculated the cortisol increment (ΔF) as (stimulated cortisol − baseline cortisol) (μg/dL).

Data collection

In all patients we recorded the following data at study entry: age, sex, admission diagnosis, focus of infection, and 28-day mortality. We also recorded septic status (septic shock or not) and severity of illness at study entry and on each day of blood sampling according to Acute Physiology and Chronic Health Evaluation (APACHE) II and Sequential Organ Failure Assessment (SOFA) scoring systems (16, 17).

Hormone and cytokine assays

Plasma cortisol was determined by an immunochemiluminescence method (ICMA-ADVIA Centaur CP Immunoassay System). ACTH was measured by immunoradiometric assay (Cis bio international). CBG was measured by a radioimmunometric assay (DRG International) (normal range: males, 27.1–52.3 mg/L; females, 31.0–53.4 mg/L).

Cytokine measurements were performed by flow cytometry (Cytometric Bead Array) technology (18). Human Inflammation Cytometric Bead Array kit (BDBiosciences) was used to quantitatively measure IL-8, IL-1β, IL-6, IL-10, TNF-α, and IL-12p70 levels. Samples were analyzed using a BD FACS Calibur flow cytometer (BDBiosciences).

Statistical analysis

We used IBM SPSS statistical package, version 20 (IBM Software Group), and GraphPad Prism, version 5.0 (GraphPad Software).

Data are presented as the mean value ± SD of the mean,. Normality was checked with the Shapiro-Wilk test. For between-group comparisons we used unpaired *t* test, Mann-Whitney *U* test, χ^2 analysis or Fisher exact test, where appropriate and for

within-group comparisons, the paired *t* test. Spearman and Pearson correlation coefficients assessed the associations between variables. Mixed-effect models were used to describe the time progression of variables (transformed in a logarithmic scale). Models included outcome during ICU stay (survivors/non survivors) or sepsis status (no shock vs shock), time and their interaction as covariates. To control for the effect of multiple factors, linear regression and general linear model analysis (patients without shock were assigned a score of 0 and patients with shock a score of 1) were used. The ratio of the adjusted values in patients with septic shock to the adjusted values in patients without shock was calculated from the antilogarithm of the coefficient for group for log-transformed cortisol levels. We used Generalized Estimating Equations to fit a repeated measures logistic regression to longitudinal binary data (patients without adrenal dysfunction were assigned a score of 0 and patients with adrenal dysfunction a score of 1). We calculated the odds ratio for death according to septic status (no shock vs shock) with Logistic Regression analysis. Differences were considered significant at $P < .05$.

Results

Study population

Fifty-one patients (39 men) with a mean age of 55 ± 18 years were included in the study. Admission diagnoses included postoperative cases (n = 25), multiple trauma (n = 15), and medical patients (n = 11). Clinical details of the study population are presented as Supplemental Results. Mean APACHE II and median SOFA scores at study entry were 19 ± 6 (range, 5–33) and 10 (range, 4–20), respectively. Overall, 19 patients died, yielding a mortality rate of 37%. The number of patients evaluated at each time point was as follows: day 1 (n = 51), day 4 (n = 51), day 7 (n = 47), day 10 (n = 34), day 13 (n = 25), day 16 (n = 21), day 21 (n = 17), day 26 (n = 14), and day 30 (n = 6).

Clinical severity and cytokine levels

Table 1 shows the clinical characteristics and the hormone and cytokine levels at study entry in septic patients with and without shock. Patients who presented with septic shock had higher APACHE II, SOFA score, IL-8, IL-6, and IL-10 levels and were more likely to die, compared with patients without shock (estimated odds ratio, 4.8; 95% CI, 1.4–17.1; $P = .02$). APACHE II and SOFA scores decreased from day 7 onward ($P = .001$ and $P < .001$, respectively). Cytokine levels also decreased during the observation period ($P < .001$, for all) (Figure 1).

Patients admitted with septic shock retained higher APACHE II ($P < .001$), SOFA score ($P < .001$), IL-6 ($P = .02$), and IL-10 ($P = .01$) levels at several time points compared with those without. The two groups had comparable IL-1β, TNF-α, and IL-12p70 levels (all P = nonsignificant [NS]).

Table 1. Clinical Characteristics, Hormone, and Cytokine Levels in Septic Patients without Shock and Those with Shock at Study Entry (mean ± SD)

	No Shock (n = 35)	Shock (n = 16)	*P* Value
Age, y	50 ± 17	66 ± 17	.05
Sex, M/F	32/3	7/9	.001
Survivors/ nonsurvivors	26/9	6/10	<.01
APACHE II	17 ± 6	25 ± 5	<.001
SOFA	9 ± 3	14 ± 3	<.001
Baseline cortisol, μg/dL	17 ± 5	20 ± 6	.02
Stimulated cortisol, μg/dL	23 ± 6	26 ± 5	.04
ΔF, μg/dL	6 ± 5	6 ± 3	NS
ACTH, pg/mL	29 ± 24	19 ± 12	NS
IL-1β, pg/mL	100 ± 177	78 ± 91	NS
IL-6, pg/mL	601 ± 1146	1468 ± 1861	<.01
IL-8, pg/mL	226 ± 332	529 ± 1202	.04
IL-10, pg/mL	24 ± 54	129 ± 266	.01
IL-12p70, pg/mL	19 ± 23	14 ± 16	NS
TNF-α, pg/mL	3 ± 3	4 ± 6	NS

Clinical characteristics and hormone and cytokine levels at study entry in survivors and nonsurvivors are shown in Supplemental Table 1. Although there was no difference in APACHE II score, nonsurvivors were in a more critical state as reflected by the higher SOFA score. Furthermore, nonsurvivors had higher IL-6, IL-8, and IL-10 levels upon development of sepsis. In mixed-model analysis nonsurvivors retained higher APACHE II ($P < .001$) and SOFA scores ($P < .001$), IL-6 ($P < .001$), and IL-8 ($P < .001$) levels, at several time points on subsequent measurements. IL-10 concentrations were higher in nonsurvivors ($P = .0001$) only at study entry. The two groups had comparable IL-1β, TNF-α, and IL-12p70 levels (all P = NS).

Course of baseline cortisol and ACTH levels

Baseline cortisol values ranged between 4.2–42.5 μg/dL with no significant intra-individual variability during the entire observation period (P = NS) (Figure 2A) and no differences between medical, surgical, and acute trauma patients (Supplemental Table 2). Baseline FCI, decreased significantly in the chronic phase (from Day 10 onward), whereas CBG levels increased (Figure 2B,C). Initial ACTH levels varied between 3.4 pg/mL and 120 pg/mL and increased significantly in the chronic phase of sepsis ($P < .0001$) (Figure 2D).

Nonsurvivors had higher baseline cortisol at all time points in ($P < .001$), but ACTH levels were similar to survivors. In Receiver Operating Characteristic (ROC) curve analysis a baseline cortisol above 18 μg/dL on day 1 had 60% sensitivity and 70% specificity for predicting mortality (area 0.71, $P = .02$).

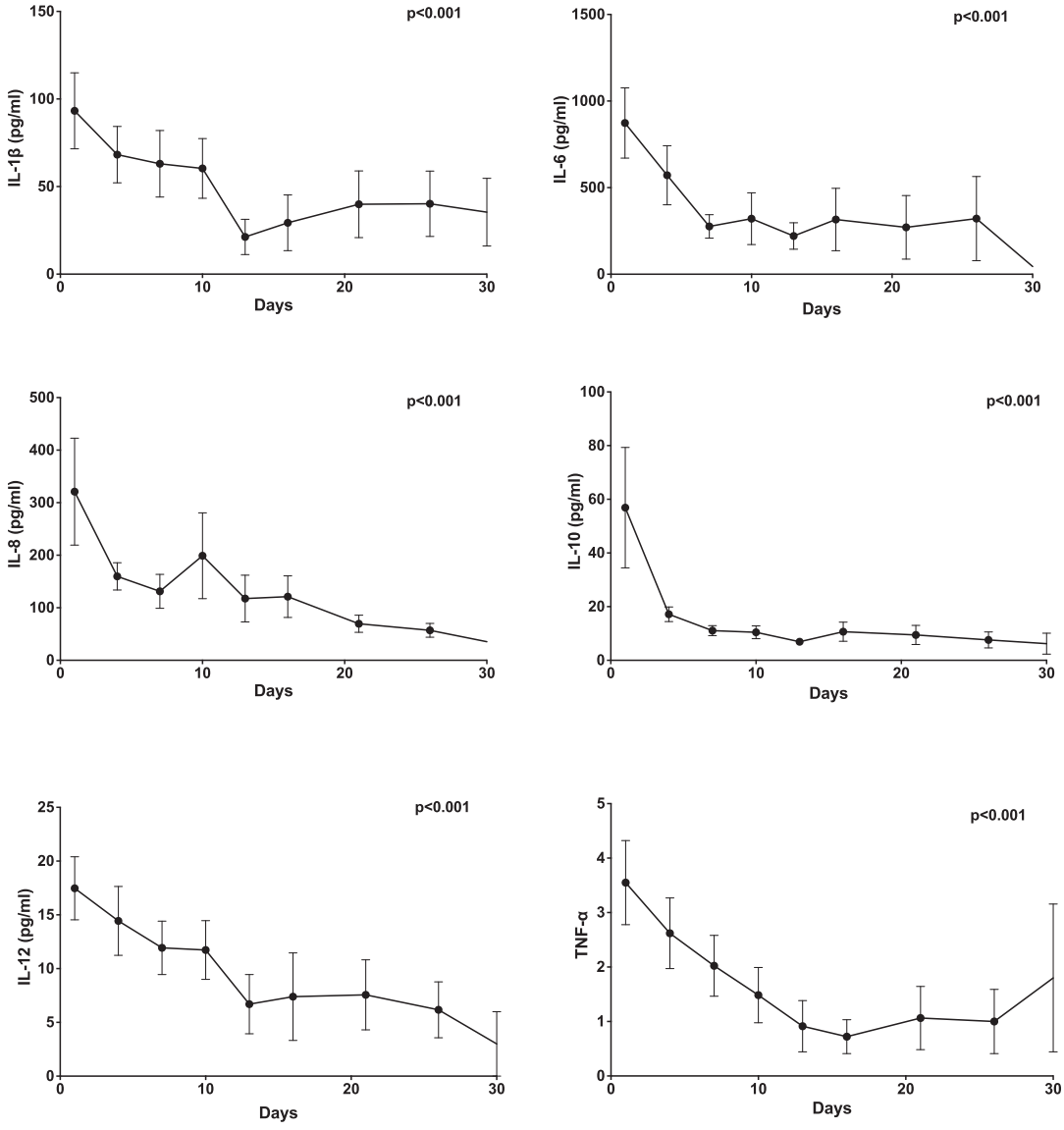

Figure 1. Course of cytokine levels in the whole cohort during the observation period.

Relationship between baseline cortisol and ACTH levels

On Day 1, ACTH levels could not predict cortisol levels ($R^2 = 0.06$, $P = .08$). General Linear Model analysis adjusting for ACTH levels showed that, for a given ACTH, patients without shock had lower baseline cortisol levels compared with patients with shock by a factor of 0.77 ($P = .01$).

In the chronic phase, sepsis status was not significant in predicting cortisol levels, but ACTH levels could predict 35% and 49% of the variation in cortisol levels on days 10 and 14 ($R^2 = 0.35$, $P < .001$; and $R^2 = 0.49$, $P < .001$, respectively) (Figure 3).

Inclusion of cytokine levels (IL-6, IL-8, IL-10, and TNF-α) in the models did not alter the results.

Course of stimulated cortisol levels

Cortisol levels increased significantly 30 minutes after 1 μg ACTH 1–24 iv at all time points (all $P < .01$). In the whole cohort, stimulated total cortisol levels, or ΔF had no significant longitudinal variation (Figure 2, E and F), with no differences between medical, surgical, and acute trauma patients (Supplemental Table 2).

On Day 1, stimulated cortisol levels were similar between survivors and nonsurvivors resulting in a lower difference between stimulated and baseline cortisol (ΔF) in nonsurvivors (7.2 $\pm$ 4.4 μg/dL vs 3.9 $\pm$ 2.7 μg/dL, $P = .04$). In ROC curve analysis, an increment (ΔF) of <5.7 μg/dL on day 1 had a 73.3% sensitivity, and a 63.3% specificity, for predicting mortality (area 0.72, $P = .02$). In subsequent measurements nonsurvivors had higher stim-

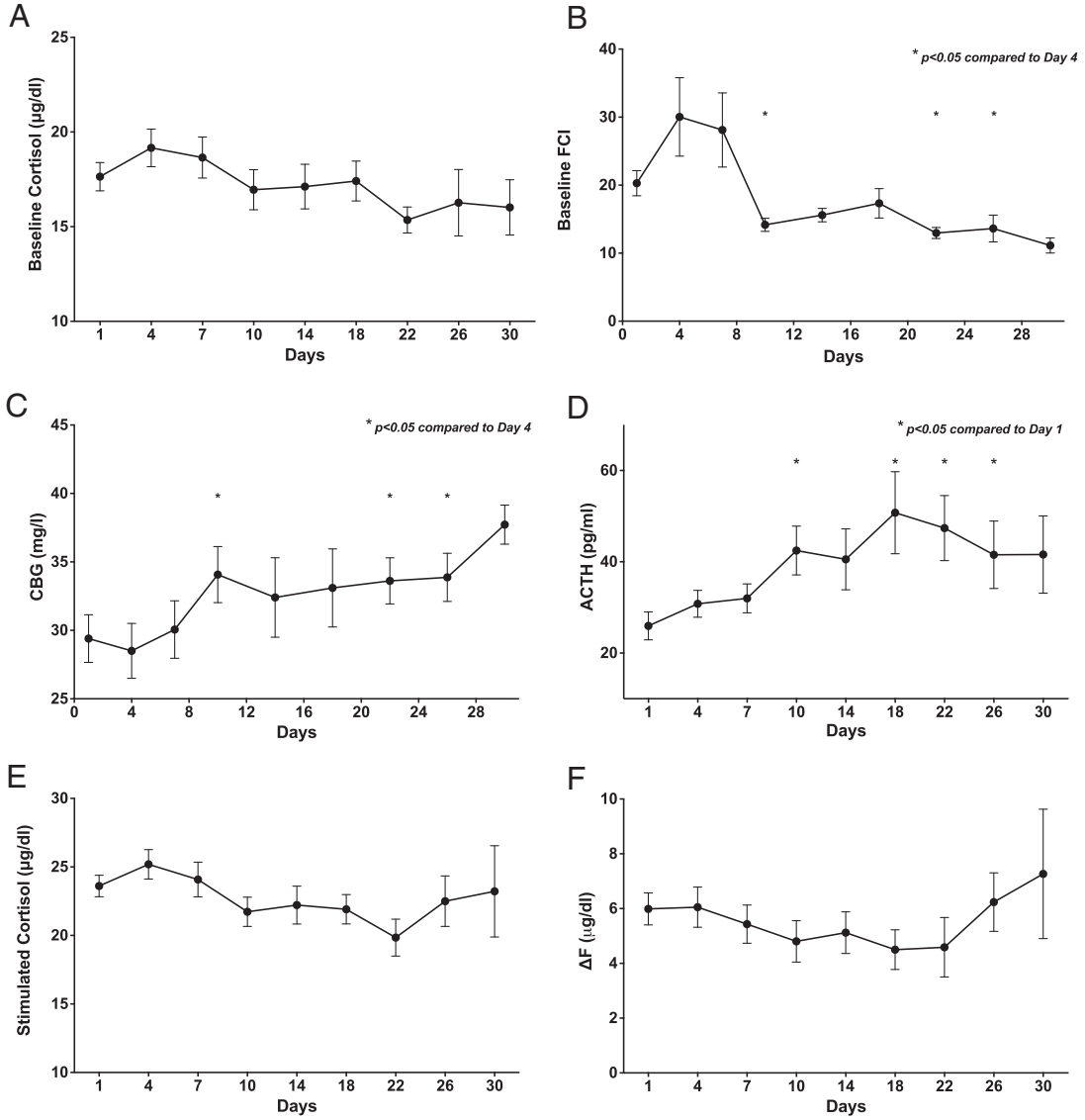

Figure 2. Course of A, baseline cortisol; B, FCI; C, CBG; D, ACTH levels; E, stimulated cortisol; and F, ΔF in the whole cohort during the observation period.

ulated cortisol levels compared with survivors ($P = .01$) with no difference in ΔF.

Characteristics of patients with septic shock

Patients who presented with septic shock had significantly higher baseline and stimulated total cortisol levels, compared with patients who were not in shock initially, both at entry (Table 1) and throughout the study period ($P = .036$ and $P = .015$, for mixed-model analysis of baseline and stimulated total cortisol, respectively). There was no difference in ΔF or ACTH levels.

A number of patients changed their sepsis stage during the course of their illness. When sepsis stage at each time point was considered, patients with septic shock had higher baseline and stimulated cortisol at several time points, but similar ΔF and ACTH levels compared with patients without shock (Supplemental Table 3).

Twelve patients developed septic shock during the study period. Compared with the values obtained before septic shock, no significant change in their basal or stimulated cortisol levels, ΔF (Figure 4A), or ACTH levels was noted. In twelve patients septic shock was reversed during the course of their illness; basal and stimulated cortisol levels, ΔF (Figure 4B), and ACTH levels at the time of septic shock and after its resolution were not significantly different. There were also no changes to baseline FCI. An initial baseline cortisol > 18 μg/dL was associated with increased risk of subsequently developing septic shock with a hazard ratio of 6.2 (95% CI, 1.2–30.1).

Adrenal dysfunction

Table 2 shows the percentage of patients with adrenal dysfunction according to various criteria. In generalized estimating equations analysis no specific time point was

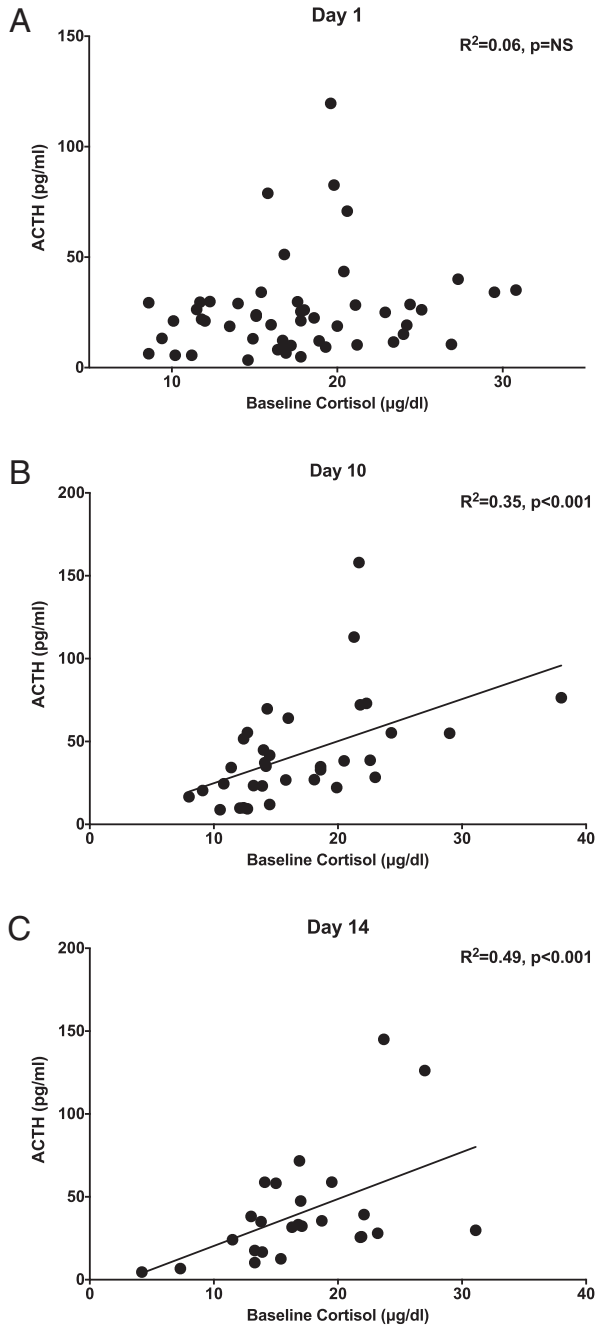

Figure 3. Correlation between ACTH levels and basal cortisol levels on Days A, 1; B, 10; and C, 14.

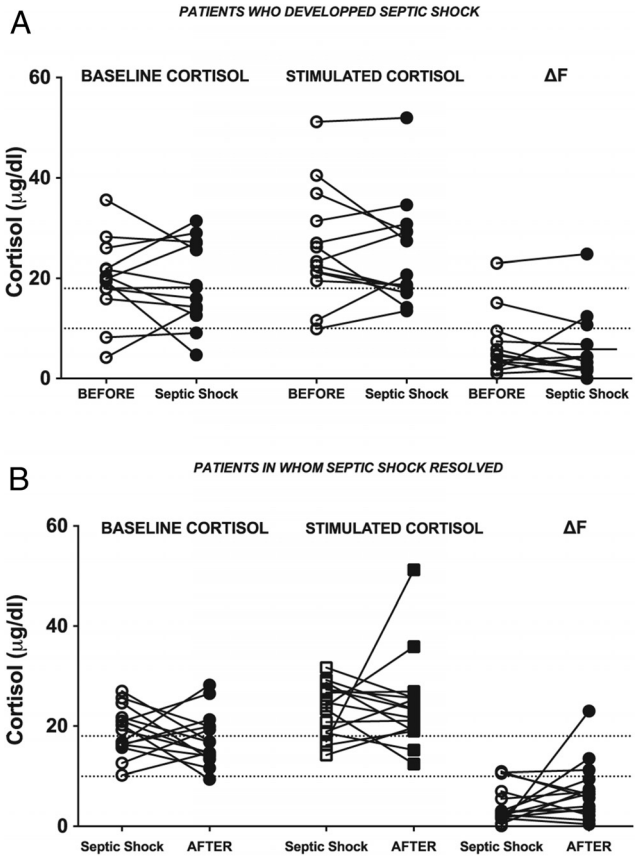

Figure 4. A, Basal, stimulated cortisol levels and ΔF before and after development of septic shock. B, Basal, stimulated cortisol levels, and ΔF during septic shock and after its resolution.

associated with a higher probability of adrenal dysfunction defined by various criteria: ΔF ≤ 4 μg/dL or 9 μg/dL, baseline cortisol ≤ 10 μg/dL, 15 μg/dL, 18 μg/dL, 20 μg/dL, 25 μg/dL, or 34 μg/dL; and stimulated cortisol ≤ 18 μg/dL or 25 μg/dL. Only day 10 was associated with a higher probability of ΔF < 5.7 μg/dL ($P = .02$). The estimated correlations for within-subject measurements were not consistent, ranging from <0.001 to 0.58, for subsequent time points. There was no specific pattern in the responses and many patients changed classification, between having and not having adrenal dysfunction, each time they were tested. None of the three patients with initial baseline cortisol ≤ 10 μg/dL, continued to do so. Most of the 48 patients with initial baseline cortisol levels > 10 μg/dL, remained above this threshold expect for eight patients; three fluctuated, whereas five had consistently low baseline cortisol in the late phase of sepsis. Among those with stimulated levels ≤ 18 μg/dL, three had consistently low levels, four had higher values when they were retested, and in two patients the levels fluctuated above and below this threshold. Among those with stimulated total cortisol levels > 18 μg/dL, 31 had consistently high levels, whereas the others had lower or higher levels on each retesting.

Discussion

In this study we sought to systematically characterize basal and stimulated cortisol levels in a fairly large cohort of intubated septic patients, covering both the acute and chronic phases of critical illness. We used the low dose, 1 μg, ACTH 1–24 test, a more sensitive test to identify adrenal atrophy as opposed to the standard 250-μg dose. Our main finding was that both basal and stimulated total cortisol levels do not

Table 2. Percentage of Adrenal Dysfunction at Each Time Point, According to Various Criteria

Criterion	Day 1	Day 4	Day 7	Day 10	Day 14	Day 18	Day 22	Day 26	Day 30
Baseline F $<$ 10 μg/dL	6	8	6	6	8	0	0	14	0
Baseline F $<$ 15 μg/dL	29	29	30	53	36	38	53	43	50
Baseline F $<$ 18 μg/dL	59	47	66	59	64	57	82	64	67
Baseline F $<$ 20 μg/dL	71	63	75	71	72	76	88	71	83
Baseline F $<$ 25 μg/dL	90	82	81	94	92	91	100	86	100
Baseline F $<$ 34 μg/dL	100	92	94	97	100	100	100	100	100
Stimulated F $<$ 18 μg/dL	18	14	17	32	32	24	41	29	17
Stimulated F $<$ 25 μg/dL	63	61	64	74	68	76	88	71	67
ΔF $<$ 4 μg/dL	39	35	43	50	44	52	53	43	17
ΔF $<$ 5.7 μg/dL	51	59	66	79	56	62	65	50	33
ΔF $<$ 9 μg/dL	77	82	85	91	88	86	88	79	83

vary significantly during the course of sepsis. Rather, cortisol levels were to a great extent related to the severity of initial insult, as demonstrated by the higher baseline and stimulated cortisol levels in those with septic shock, compared with those with less severe sepsis status. Nevertheless, despite the lack of significant variations in baseline and stimulated total serum cortisol levels, the free cortisol index (FCI) that represents a fair estimate of serum free cortisol showed significantly higher values in the early compared with the late phase of critical illness. A remarkable finding was that ACTH levels showed a significant late increase that mirrored the decline in cytokine levels. Finally, from a clinical practice standpoint when different published criteria were applied at different time points of this study population, there was much inpatient variability in the diagnosis of adrenal dysfunction.

To our knowledge, this is the first prospective study that systematically investigated both baseline and stimulated cortisol levels, on multiple measurements, for a prolonged period of time (up to 30 days), in a relatively large cohort of septic patients. Our data suggest that serum cortisol levels are related to the severity of the initial stressor, in agreement with previous studies (19, 20) reporting single cortisol measurements, where high plasma cortisol levels were reproducibly associated with increased mortality (4). We additionally showed that higher levels persist throughout the course of critical illness. Thus, our findings support that, on the basis of total serum cortisol measurements, there is no specific time point of a major change during the course of critical illness. Rather, both the baseline and stimulated cortisol levels characterized each subject from the initial event and remained fairly stable during the course of sepsis, both in survivors and nonsurvivors. Patients with initially higher levels retained these levels and were more likely either to have more severe disease (septic shock), or to develop septic shock later on, and were also more likely to die. Nonsurvivors, and in particular those with septic shock, had higher baseline cortisol levels that remained high throughout. So far, only a few studies assessed unstimulated cortisol levels longitudinally, focusing in the acute, up to 10 days, phase of critical illness (4, 9–13). One study reached to 14 days (10). Most of these studies reported constantly increased plasma cortisol levels. One that extended to the chronic phase (21) reported decreasing cortisol levels in survivors during late sepsis.

A remarkable finding of this study was that although ACTH levels were decreased initially they raised significantly later on, mirroring the decline in cytokine levels. Importantly, ACTH levels during acute sepsis were not related to cortisol levels. This relationship became significant during late sepsis. Vermes et al (12) have also reported dissociation between cortisol and ACTH levels during the acute phase of critical illness. According to our data, ACTH is probably not the main determinant of cortisol levels during the early phase of critical illness. This pituitary-independent adrenal stimulation is in accordance with previous studies and may be caused by massively increased cytokines, neuropeptides, adipokines, and other factors that have been shown to act directly on the adrenals (12, 14). Another contributing factor is decreased peripheral cortisol clearance (22). In a recent study (9), critically ill patients had significantly higher cortisol levels but lower ACTH levels compared with controls. Moreover, there was a trend for increased ACTH levels over the 7 days of observations, in agreement with our results with a longer follow-up. The most likely explanation is that during prolonged sepsis, cytokine levels subside and ACTH regains control to maintain cortisol at the appropriate levels, as supported by the finding that at this time point ACTH levels correlated with cortisol levels. Thus, in the chronic phase ACTH takes over as a major adrenal stimulant.

One of the recently posed questions is whether adrenal dysfunction occurs late in the course of critical illness and relates to adrenal atrophy caused by low ACTH levels in the acute phase (9). Our data do not support the development of adrenal atrophy, given that we did not observe gradually reduced responses to the low-dose ACTH 1–24 test. It is probable that adrenal gland integrity is maintained by the action of cytokines even in the face of reduced ACTH levels. Stimulation of the adrenals with synthetic ACTH has become a standard component of the testing

procedures applied for the assessment of adrenocortical function in critically ill patients (23), but there is limited data with regard to repeated testing during the course of critical illness. In the few previous studies only two tests were performed, with poor reproducibility and questionable clinical utility (11, 24–26). In the present study stimulated cortisol levels by multiple low-dose ACTH 1–24 challenges during the whole course of critical illness showed no significant differences in cortisol responses. Interestingly we observed increased stimulated cortisol levels in the more severely ill patients, ie, those with septic shock and those who did not survive. Baseline, stimulated cortisol levels, or ΔF did not change upon development of septic shock, indicating that this complication was not the result of alteration of the patients' adrenal status.

An ongoing controversial issue is the cut-offs for baseline or stimulated cortisol levels that define adrenal dysfunction. In view of the lack of unequivocal clinical manifestations of adrenal insufficiency in the setting of critical illness, a robust definition of normal adrenal response is unachievable. So far, several criteria have been introduced (2, 3). The prevalence of adrenal dysfunction varied according to the applied criteria, in accordance with numerous previous studies. Importantly, the higher the applied cut-offs the higher was the prevalence of alleged adrenal dysfunction. Although the variation in cortisol levels was not significant, it was sufficient enough to result in a change of classification, between having and not having adrenal dysfunction, in many patients, each time they were tested. The change in classification did not have a specific pattern and we were unable to identify a time point of adrenal dysfunction. Thus, whether the observed change in the responses is true or just a random variation cannot be documented. Overall, these findings illustrate the difficulties to obtain a robust diagnosis of adrenal dysfunction based solely on biochemical data.

The low-1 μg dose, used in the present study, has been suggested to increase the sensitivity for the diagnosis of adrenal insufficiency in patients with secondary adrenal failure. This test, however, has only rarely been assessed in critically ill patients (27–30). In head-to-head comparison studies with the 250-μg (high) dose (28, 29), the nonresponders to the low dose were greater than to the high dose test. The most commonly used cut-off for the 250 μg dose is the $\Delta F \leq 9$, given that it has been reported to be associated with increased mortality, but unlike Annane et al (20) we did not test all patients shortly after the development of shock, but within 72 hours (most patients were investigated within 24 h). Nevertheless, we evaluated adrenal profile both before and after shock development and showed that the development of septic shock was not associated with a change in the adrenal dynamics. Data for the appropriate cut-off level for the low-dose ACTH 1–24 test in the diagnosis of adrenal dysfunction are limited. Application of the widely accepted cut-off for the high-dose ACTH 1–24 test of 9 μg/dL (20) cannot be extrapolated to the low-dose test. Peak cortisol responses of less than 18 μg/dL may be more relevant. We also used a lower cut-off for ΔF of 6 μg/d, which derived from a ROC curve predicting mortality. It should be noted, however, that we applied this test principally as a sensitive tool to detect the hypothesized late adrenal atrophy, rather than to establish its diagnostic use.

A limitation of our study is that only total cortisol levels were used since this method is more widely available and the vast majority of existing data are based on such measurements. Due to marked decreases in cortisol binding, total cortisol measurements may not reflect changes in free cortisol, which represents the active component that diffuses to target tissues. In the present study, in accordance with previous data (10), FCI values were higher in the early compared with the late phase of sepsis. Boonen et al recently published similar findings for serum free cortisol levels during the acute phase (9). The higher free cortisol estimated levels are due to decreased CBG levels during the acute phase of sepsis, allowing more cortisol to act to its targets. In fact, calculated free cortisol levels may be underestimated. Decreased albumin levels and changes in the binding capacity of CBG represent additional mechanisms that increase the cortisol free fraction in the acute phase of critical illness. Coolens' method (31), which takes into account the albumin levels, may be superior (32, 33); however, albumin measurements corresponding to all timepoints of blood sampling was not available and therefore FCI was used as a reasonable alternative (10, 34). However, both intact and cleaved (unable to bind cortisol) CBG may coexist in the circulation (35) and in sepsis, where cleaved CBG is expected to predominate, the calculated free cortisol levels may be erroneously low. These data suggest that free cortisol may be a more appropriate measure for assessing adrenal integrity in the acute phase. Measurements of serum free cortisol, however, are not widely available and so far there are no widely accepted cut-offs for either free cortisol or FCI. Moreover, we have recently demonstrated that in sepsis interstitial cortisol levels correlate only moderately both with total and free plasma cortisol, suggesting that plasma cortisol may not reflect tissue availability (36).

In conclusion, during sepsis, total cortisol levels relate both to the severity of the initial insult and the outcome and remain fairly unchanged during the course of illness. Initially, the adrenal production of cortisol is largely due to non-ACTH dependent factors, whereas ACTH gains control later on, as the inflammatory cascade subsides and

the levels of these factors decline. The development of septic shock does not seem to be due to adrenal exhaustion, whereas adrenal dysfunction, related to atrophy due to the initially low ACTH levels, does not seem to be a major problem during the prolonged phase of sepsis, at least when total cortisol levels are considered. Although not significant, the variation in cortisol levels was such that the classification of the patients was highly variable, questioning the utility and interpretation of the results of ACTH 1–24 testing in critically ill patients.

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