Diagnosis of Adrenal Insufficiency in Severe Sepsis and Septic Shock

Djillali Annane, Virginie Maxime, Fidaa Ibrahim, Jean Claude Alvarez, Emuri Abe, and Philippe Boudou

Service de Réanimation and Service de Biochimie-Pharmacologie, Hôpital Raymond Poincaré, Faculté de Médecine Paris Ile de France Ouest, Garches; and Service de Biochimie Hormonale, Hôpital Saint Louis, Faculté de Médecine Saint Louis Lariboisière, Paris, France

Rationale: **Diagnosis of adrenal insufficiency in critically ill patients has relied on random or cosyntropin-stimulated cortisol levels, and has not been corroborated by a more accurate diagnostic standard.**
Objective: **We used the overnight metyrapone stimulation test to investigate the diagnostic value of the standard cosyntropin stimulation test, and the prevalence of sepsis-associated adrenal insufficiency.**
Methods: **This was an inception cohort study.**
Measurements and Results: **In two consecutive septic cohorts (n = 61 and n = 40), in 44 patients without sepsis and in 32 healthy volunteers, we measured (*1*) serum cortisol before and after cosyntropin stimulation, albumin, and corticosteroid-binding globulin levels, and (*2*) serum corticotropin, cortisol, and 11β-deoxycortisol levels before and after an overnight metyrapone stimulation. Adrenal insufficiency was defined by postmetyrapone serum 11β-deoxycortisol levels below 7 μg/dl. More patients with sepsis (31/61 [59% of original cohort with sepsis] and 24/40 [60% of validation cohort with sepsis]) met criteria for adrenal insufficiency than patients without sepsis (3/44; 7%) ($p < 0.001$ for both comparisons). Baseline cortisol (< 10 μg/dl), Δ cortisol (< 9 μg/dl), and free cortisol (< 2 μg/dl) had a positive likelihood ratio equal to infinity, 8.46 (95% confidence interval, 1.19–60.25), and 9.50 (95% confidence interval, 1.05–9.54), respectively. The best predictor of adrenal insufficiency (as defined by metyrapone testing) was baseline cortisol of 10 μg/dl or less or Δ cortisol of less than 9 μg/dl. The best predictors of normal adrenal response were cosyntropin-stimulated cortisol of 44 μg/dl or greater and Δ cortisol of 16.8 μg/dl or greater.**
Conclusions: **In sepsis, adrenal insufficiency is likely when baseline cortisol levels are less than 10 μg/dl or delta cortisol is less than 9 μg/dl, and unlikely when cosyntropin-stimulated cortisol level is 44 μg/dl or greater or Δ cortisol is 16.8 μg/dl or greater.**

Keywords: corticol injection; free cortisol; corticotropin

Almost one century after the original description of apoplexy of the adrenal glands in septic shock (1), consensus is lacking on diagnostic criteria to define adrenal insufficiency in critical illness (2). In unstressed subjects, adrenal insufficiency is defined by a cosyntropin-stimulated cortisol level less than 18–20 μg/dl (3, 4). In critical illness, the diagnostic criteria for adrenal insufficiency have included a random cortisol level lower than 15 (2) or 25 μg/dl (5), or a cortisol increment after cosyntropin stimulation of 9 μg/dl or less (2, 6). In patients with severe hypoproteinemia, adrenal insufficiency may be defined by serum free cortisol level of less than 2.0 μg/dl at baseline or less than 3.1 μg/dl after cosyntropin stimulation (7). We have recently provided data underscoring the clinical significance of adrenal insufficiency in patients with septic shock (8–11). After 250 μg cosyntropin stimulation, patients with a cortisol increment of 9 μg/dl or less (nonresponders to cosyntropin) had vasopressor hyporesponsiveness (8), higher risk of death (9), and improved response to prolonged corticosteroid supplementation (10, 11). However, that nonresponders to cosyntropin had adrenal insufficiency remains controversial.

In fact, the use of the cosyntropin stimulation test to assess adrenal function may present some variability (12), and may lead to misdiagnosing secondary adrenal insufficiency (13). More sensitive and cumbersome reference tests, such as insulin tolerance and metyrapone stimulation, have not been evaluated in intensive care unit (ICU) patients (2). Today, the use of insulin tolerance is impractical, because intensive insulin therapy has become a standard of care for ICU patients (14, 15), and septic shock is commonly associated with peripheral insulin resistance. For this reason, we used the overnight single-dose metyrapone stimulation test to investigate the diagnostic value of the standard 250-μg cosyntropin stimulation, and the prevalence of sepsis-associated adrenal insufficiency.

AT A GLANCE COMMENTARY

Scientific Knowledge on the Subject

Adrenal insufficiency may be a frequent complication of critical illnesses such as severe sepsis, and may be associated with a worse outcome. Its diagnosis remains controversial.

What This Study Adds to the Field

Changes in serum total or free cortisol after corticotropin bolus can be used to detect adrenal insufficiency in septic patients.

METHODS

Study Population

The CCPPRB de St. Germain en Laye approved the study, and healthy volunteers and patients or their relatives provided written informed consent before enrolment.

Patients with Sepsis

All consecutive ICU patients were prospectively included if they had severe sepsis or septic shock (16). Patients who were enrolled from

(*Received in original form September 3, 2005; accepted in final form September 8, 2006*)

Supported by a grant from Délégation Régionale à la Recherche Clinique, Ile de France–Assistance Publique–Hôpitaux de Paris (Ger-Inf-05R2 from Groupe d'Etude et Recherche sur le Médicament [GERMED]).

The study sponsor had no responsibility for the study design, data analysis and interpretation, or decision to submit this manuscript for publication.

Correspondence and requests for reprints should be addressed to Djillali Annane, M.D., Ph.D., Service de Réanimation, Hôpital Raymond Poincaré (AP-HP), Faculté de Médecine Paris Ile de France Ouest (UVSQ), 104 Boulevard Raymond Poincaré, 92380 Garches, France. E-mail: djillali.annane@rpc.aphp.fr

This article has online supplement, which is accessible from this issue's table of contents at www.atsjournal.org

Am J Respir Crit Care Med Vol 174. pp 1319–1326, 2006
Originally Published in Press as DOI: 10.1164/rccm.200509-1369OC on September 14, 2006
Internet address: www.atsjournals.org

February 2002 to May 2004 constituted the original cohort with sepsis, and those recruited from December 2005 to April 2006 constituted the validation cohort with sepsis.

ICU Patients with No Sepsis

From December 2005 to April 2006, ICU patients without sepsis who were expected to have intact adrenal function and a short ICU stay served as control subjects.

Exclusion criteria for patients with sepsis and those without sepsis were as follows: age of less than 18 yr; pregnancy or breast-feeding; history of infection with human immunodeficiency virus; any known preexisting endocrine or liver disease (including any stage of cirrhosis, acute or chronic viral hepatitis, alcoholic liver disease, or hepatic tumors); any treatment with etomidate, glucocorticoids, estrogen, or any drug interfering with the hypothalamic–pituitary adrenal axis in the preceding 6 mo (4, 17).

At study entry, the following parameters were recorded: time from ICU admission; age and sex; past medical history and estimated prognosis of any underlying disease, stratified according to the criteria of McCabe and Jackson (0, nonfatal; 1, ultimately fatal [i.e., < 5 yr]; or 3, rapidly fatal [i.e., < 1 yr]) (18); severity of illness, as assessed by the Simplified Acute Physiology Score (SAPS) II (scores can range from 0 to 163, with higher scores indicating higher risk of death) (19); the Sepsis-Related Organ Failure Assessment (SOFA) score (score can range from 0 to 24, with scores for each organ system [respiratory, hematological, hepatic, cardiovascular, neurological, and renal] ranging from 0 [normal] to 4 [most abnormal]) (20); and vital signs. Laboratory measurements included arterial blood gas, with the ratio of the partial pressure of arterial oxygen to the fraction of inspired oxygen, lactate levels, blood and urinary electrolytes concentration, total white blood cells, eosinophil and platelet counts, and serum levels of albumin and glucose.

On ICU admission, patients had blood samples drawn before and 60 min after a 250-μg cosyntropin administration. Later that day (> 8 h), patients received an overnight single-dose metyrapone stimulation test (30 mg/kg of the adrenal 11β-hydroxylase inhibitor, metyrapone, administered through the gastric tube at midnight during enteral nutrition); (3) blood samples were drawn before the test and at 8 A.M. the following morning. Then, 50 mg hydrocortisone was given intravenously every 6 h and 50 μg of fludrocortisone through the gastric tube once daily, or for 7 d in nonresponders to cosyntropin (10). A total of 32 healthy, sex-matched subjects (age, 16–72 yr) underwent similar tests.

Reference Standard for the Diagnosis of Adrenal Insufficiency and Nonresponders to Cosyntropin

The metyrapone stimulation test is based upon the fact that low serum cortisol levels normally stimulate secretion of the adrenocorticotropic hormone (ACTH), corticotropin. Metyrapone blocks the conversion of 11β-deoxycortisol to cortisol, the last step in the biosynthetic pathway from cholesterol to cortisol. As a result, cortisol synthesis and secretion fall while 11β-deoxycortisol accumulates in serum. Adrenal insufficiency was defined by an increment from baseline in 11β-deoxycortisol concentration of less than 7 μg/dl at 8:00 A.M. while cortisol level had fallen below 8 μg/dl (3). Those who also had a corticotropin level of less than 150 pg/ml were considered as having secondary adrenal insufficiency. We assumed that metyrapone was well absorbed, and that results could be reliably interpreted when cortisol level at 8:00 A.M. was less than 8 μg/dl and peak metyrapone level was 100 ng/ml or less. Finally, patients with a cortisol increment after cosyntropin of 9 μg/dl or less were considered to be nonresponders to cosyntropin; the remainders were considered to be responders (9)

Hormonal Assays

Cortisol and corticosteroid-binding globulin were assayed at baseline and 60 min after cosyntropin stimulation. Corticotropin, cortisol, 11β-deoxycortisol, and metyrapone were assayed at baseline and at 8:00 A.M. after metyrapone administration. Plasma corticotropin and cortisol levels were measured using chemiluminescence immunoassays (Nichols Institute Diagnostics, San Clemente, CA). The intra-assay and interassay

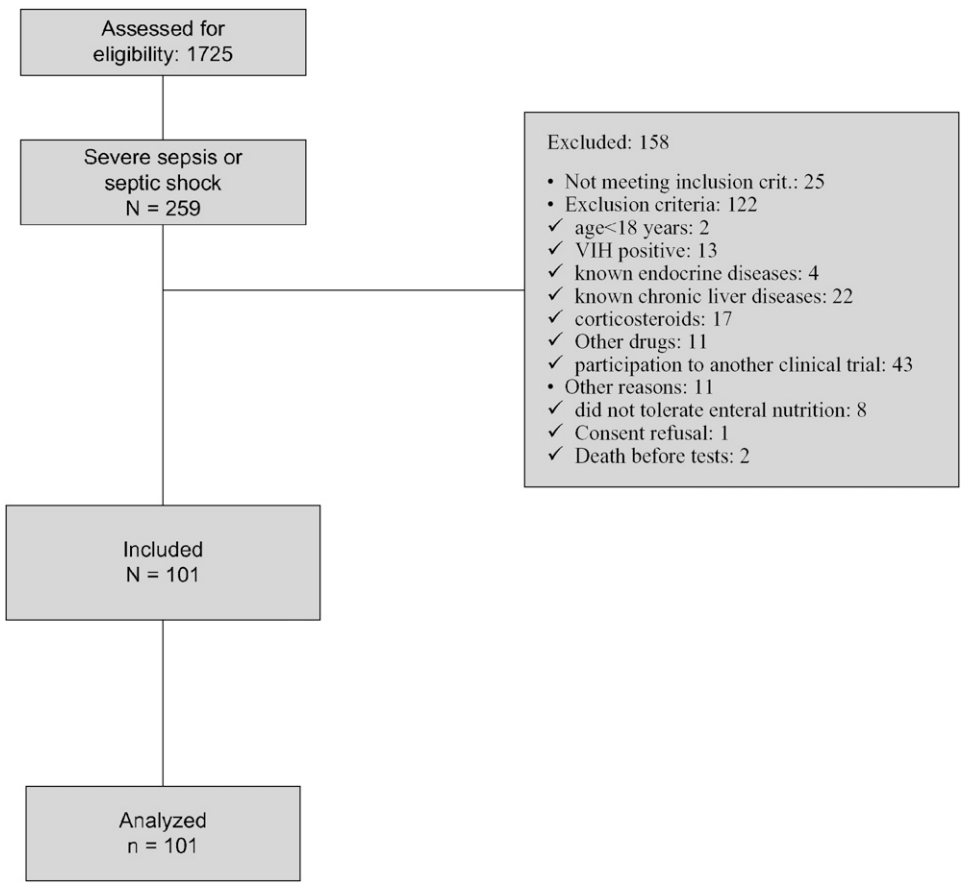

Figure 1. Flow chart of patients assessed for eligibility in the study.

coefficients of variation for corticotropin ranged from 1.2 to 4.2% and from 6.4 to 8.4%, respectively, and those for cortisol ranged from 3.0 to 3.8% and from 3.5 to 6.7%, respectively. The serum corticosteroid-binding globulin (CBG) levels were measured using a radioimmunoassay (CBG-RIA-100 kit; BioSource Europe S.A., Nivelles, Belgium), with intra-assay and interassay coefficients of variation ranging from 2.9 to 3.9% and from 2.4 to 5.5%, respectively. Serum 11β-deoxycortisol levels were measured using a specific time-resolved fluoroimmunoassay after an extraction plus celite chromatography partition step (21). The intraassay and interassay coefficients of variation ranged from 2.8 to 6.6% and from 4.6 to 7.9%, respectively. Serum levels of metyrapone were measured using liquid chromatography with tandem mass spectrometry detection. The intraassay and interassay coefficients of variation ranged from 5.3 to 8.4% and from 9.3 to 14.7%, respectively. Free cortisol levels were calculated using the Coolens method (22).

Statistical Analysis

Numeric variables were reported as medians (first and third quartiles) and categorical variables as number of patients (percentages). Variables were evaluated for an association with the diagnosis of adrenal insufficiency with the use of Pearson's χ^2 (or Fisher's exact tests) for categorical data, and the Mann-Whitney U test for numerical data. The groups were compared with the use of analysis of variance (with subsequent Bonferroni tests where appropriate) for numerical data, and the Pearson χ^2 (or Fisher's exact tests) for categorical data. The diagnostic accuracy of total and free cortisol levels, and of Δ total and free cortisol levels after cosyntropin, was tested against the results of the overnight metyrapone test. We plotted Δ and cosyntropin-stimulated values against baseline values for total and free cortisol levels. We then determined, by graphic analysis, cutoffs that best identified true negative tests (values with highest specificity) for baseline, cosyntropin-stimulated, and Δ of total and free cortisol levels. We computed the positive likelihood ratio for different cut-offs for each variable. The best test identified from the original cohort with sepsis was retested in the validation cohort with sepsis. Receiver operating characteristic curves were constructed to illustrate various cutoffs of baseline, cosyntropin-stimulated, and Δ total and free cortisol levels. We also computed a multiple stepwise logistic-regression model using the original cohort with sepsis, in which a p value of 0.15 or less was used as a criterion for entry into the model. The predictors included any clinical or laboratory findings identified by

TABLE 1. PATIENTS' MAIN CHARACTERISTICS

Variables	Original Cohort with Sepsis (*n = 61*)	Validation Cohort with Sepsis (*n = 40*)	ICU Patients without Sepsis (*n = 44*)
Age, yr	67 (52:78)	61 (47:74)	50 (37:68)*
Sex, male	41 (67)	22 (55)	26 (59)
McCabe > 0	14 (23)	15 (38)	7 (16)*†
Time from admission, d	1 (0:5)	1 (1:2)	1 (1:2)
SAPSII	48 (34:62)	41 (35:53)	33 (24:51)*†
Mean arterial pressure, mm Hg	65 (57:82)	67 (55:81)	74 (65:84)
SIRS criteria			
Heart rate, beats/min	100 (91:130)	97 (83:110)	99 (78:111)
Core temperature, °C	38.6 (37.7:39.3)	37.0 (36.0:38.0)	37.0 (37.0:37.0)*†
Leucocytes, × 10^3/mm^3	11.3 (7.9:16.1)	10.5 (5.5:18.5)	9.2 (7.3:12.0)
Signs of organ hypoperfusion			
Urine output, ml/h	60 (40:86)	56 (40:77)	65 (42:95)
Pa_{O_2}/FI_{O_2}, mm Hg	189 (120:294)	179 (115:203)	204 (150:271)
Lactate, mmol/L	1.9 (1.3:2.5)	2.0 (1.0:2.8)	1.6 (1.0:2.0)
Platelets, × 10^3/mm^3	230 (148:368)	214 (141–280)	227 (183:284)
SOFA score	9 (5:12)	8 (5:11)	6 (3:7)*†
SOFA lung	3 (2:3)	3 (2:3)	2 (2:3)
SOFA cardiovascular	3 (1:4)	3 (0:4)	0 (0:1)*†
SOFA neurological	0 (0:3)	1 (0:2)	1 (0:3)
SOFA hepatic	0 (0:0)	0 (0:1)	0 (0:0)
SOFA renal	1 (0:3)	1 (0:2)	0 (0:1)*
SOFA coagulation	0 (0:1)	0 (0:1)	0 (0:0)
Mechanical ventilation	53 (87)	30 (75)	34 (59)*†
Vasopressors	44 (72)	29 (73)	6 (14)*†
Characteristics of infection			
Positive blood cultures	22 (36)	8 (20)	0 (0)*†
Gram-negative bacteria	43 (71)	26 (65)	—
Gram-positive bacteria	24 (39)	19 (48)	—
Polymicrobial	22 (36)	15 (38)	—
Negative cultures	8 (13)	4 (10)	44 (100)*†
Site of infection			
Lung	42 (69)	26 (65)	—
Abdominal	15 (25)	9 (23)	—
Urinary tract	9 (15)	5 (13)	—
Primary septicemia	6 (10)	3 (8)	—
Other	7 (12)	2 (5)	—
More than 1 site	21 (34)	14 (35)	—
No site	2 (3)	0 (0)	—
Outcome			
Length of hospital stay, d	33 (16:48)	28 (15:48)	10 (4:25)*†
Hospital mortality	27 (44)	16 (40)	4 (9)*†

Definition of abbreviations: ICU = intensive care unit; SAPS = Simplified Acute Physiology Score; SIRS = systemic inflammatory response syndrome; SOFA = Sepsis-Related Organ Failure Assessment.

Values are median (first quartile : third quartile) or n (%).

* $p < 0.05$ for comparison between nonseptic critically ill patients and original septic shock cohort.

† $p < 0.05$ for comparison between nonseptic critically ill patients and original septic shock cohort.

a p value of less than 0.10 in univariate analysis, along with information on total and free cortisol levels. Analysis was completed with Systat 10.0 (Systat Software, Inc., San Jose, CA), and a two-tailed p value of 0.05 or less indicated statistical significance.

RESULTS

Patients Characteristics

A total of 69 patients with sepsis were eligible for inclusion in the study from February 2002 to May 2004, and 43 from December 2005 to April 2006. A total of 11 patients (8 and 3 in the first and second study periods, respectively) were excluded: 2 with refractory shock died before a cosyntropin-stimulation test could be performed, 8 did not tolerate enteral nutrition and could not receive metyrapone, and 1 did not consent (Figure 1). A total of 44 ICU patients without sepsis were enrolled, including 11 patients with drug overdoses, 9 with acute cardiogenic pulmonary edema, 5 with smoke inhalation–induced acute lung injury, 5 with status epilepticus, 4 with acute myocardial infarction, 4 with acute exacerbations of chronic obstructive pulmonary disease, 3 with keto-acidosis, and 3 with acute pulmonary embolism. As compared with patients without sepsis, patients with sepsis were older, were more likely to have fatal underlying comorbidities, mechanical ventilation, or vasopressor therapy, had higher SAPSII and SOFA scores, temperature, and hospital mortality rates, and greater lengths of stay (Table 1).

Hormonal Investigations

Metyrapone was well absorbed in all subjects, and plasma metyrapone levels (*see* Figure E1 in the online supplement) and 8:00 A.M. cortisol levels (Table 2) were comparable between patients and healthy control subjects. The two groups with sepsis had similar albumin, corticotropin, and hormonal levels (Table 2). In comparison with healthy controls, both septic and patients without sepsis had lower albumin ($p < 0.001$), CBG ($p < 0.01$), and corticotropin ($p = 0.02$) levels, higher baseline total ($p = 0.03$) and free ($p = 0.04$) cortisol levels, and higher stimulated free cortisol ($p = 0.02$). In comparison with patients without sepsis, more patients with sepsis had low albumin levels ($p = 0.01$).

More patients with sepsis (31/61 [59% from the original cohort with sepsis] and 24/40 [60% of the validation cohort with sepsis]) met the criteria for adrenal insufficiency with the metyrapone test than patients without sepsis (3/44; 7%; $p < 0.001$ for both comparisons) (Table 3). In addition, 29/36 (80%) and 16/24 (67%) patients with sepsis had secondary adrenal insufficiency, as did 1/3 (33%) patients without sepsis ($p = 0.02$ for both comparisons). Similarly, more patients with sepsis had total cortisol levels of less than 15 μg/dl than did patients without sepsis.

Among the original cohort with sepsis, in comparison with patients without adrenal insufficiency, those with adrenal insufficiency were more often vasopressor dependent ($p < 0.01$), had higher SOFA cardiovascular scores ($p = 0.02$), positive blood cultures ($p = 0.001$), gram-negative sepsis ($p < 0.01$), and greater risk of in-hospital death (relative risk, 2.66; 95% confidence interval [CI], 1.17–6.06; *see* Table E1). At baseline, patients with sepsis with adrenal insufficiency had lower free cortisol levels than patients with sepsis without adrenal insufficiency ($p = 0.04$) and patients without sepsis ($p = 0.04$; Table E2). After cosyntropin stimulation, patients with sepsis with adrenal insufficiency had lower free cortisol levels ($p = 0.03$) and lower Δ total ($p = 0.03$) and Δ free ($p = 0.04$) cortisol than those without adrenal insufficiency.

Diagnostic Value of Baseline, Cosyntropin-stimulated, and Absolute Increment of Total and Free Cortisol Levels

As cutoffs associated with the highest specificity for adrenal insufficiency, graphic analysis identified baseline total and free cortisol levels of 10 μg/dl (specificity, 1; 95% CI, 1–1) and 0.8 μg/dl (specificity, 0.95; 95% CI, 0.85–1), respectively; cosyntropin-stimulated total and free cortisol levels of 20 μg/dl (specificity, 0.96; 95% CI, 0.87–1) and 2 μg/dl (specificity, 0.90; 95% CI, 0.76–1), respectively; and cosyntropin-stimulated increments in total and free cortisol levels of 9 μg/dl (specificity, 0.96; 95% CI, 0.87–1) and 2 μg/dl (specificity = 0.84; 95% CI, 0.68–1), respectively (Figure 2). The area under the receiver operating characteristic curves were lower for baseline total and free cortisol levels (0.54 [95% CI, 0.34–0.65] and 0.59 [95% CI, 0.38–0.69],

TABLE 2. HORMONAL DATA IN PATIENTS AND HEALTHY VOLUNTEERS

Variables	Original Cohort with Sepsis (*n = 61*)	Validation Cohort with Sepsis (*n = 40*)	Critically Ill without Sepsis (*n = 44*)	Healthy Volunteers (*n = 32*)
Albumin, g/L	27 (21:34)*	39.0 (23.5:35.5)*	31 (27:37)	39 (38:41)
Albumin levels < 25 g/L	21 (34.4)*†	15 (38.5)*†	9 (21.9)*	0
Basal				
Cortisol binding globulin	29 (24:37)*	30 (23:35)*	34 (25:46)	43 (36:46)
Cortisol, μg/dl	16.4 (13.0:38.8)*	17.8 (12.2:29.5)*	27.8 (19.8:34.6)*	12.6 (11.5:14.0)
Free cortisol, μg/dl	1.9 (0.6:4.6)*	1.5 (1.0:4.0)*	2.0 (1.0:5.0)*	0.7 (0.6:0.9)
11β-deoxycortisol, μg/dl	0.030 (0.010:0.124)	0.098 (0.040:0.175)	0.095 (0.051:0.250)	0.085 (0.040:0.120)
ACTH, pg/ml	12 (6:16)*	8 (4:18)*	6 (3:13)*	33 (28:37)
60 min after 250 μg ACTH				
Cortisol binding globulin	30 (23:37)*†	28 (22:44)*	35 (26:47)	43 (38:48)
Cortisol, μg/dl	30.0 (22.5:43.7)	33.5 (13.0:47.0)	36.0 (24.0:58.5)	27.4 (25.9:28.6)
Free cortisol	5.2 (3.1:9.7)*	3.9 (2.6:7.0)*	4.0 (1.3: 8.8)*	2.6 (2.3:3.2)
Δ cortisol, μg/dl	12.5 (9.1:17.1)	11.6 (3.3:30.3)	12.5 (6.1:22.7)	14.5 (13.5:15.5)
Δ free cortisol	2.6 (1.1:4.7)	1.9 (0.4:4.0)	1.0 (0.0:3.0)	1.8 (1.7:2.2)
8 h after metyrapone (8 A.M.)				
ACTH, pg/ml	83 (25:178)	83 (34:244)	143 (52:304)	165 (147:253)
11β-deoxycortisol, μg/dl	4.6 (1.1:12.2)	5.0 (2.0:13.1)	15.5 (12.0:21.0)	13.9 (10.8:18.8)
Cortisol, μg/dl	1.9 (1.0:6.1)	2.0 (1.0:5.5)	2.2 (1.3:5.7)	2.1 (1.2:6.1)

Definition of abbreviation: ACTH = adrenocorticotropic hormone.
Values are median (first quartile : third quartile) or n (%).
* $p < 0.05$ for comparison with healthy volunteers.
† $p < 0.05$ for comparison with critically ill patients without sepsis.

TABLE 3. ADRENAL FUNCTION IN PATIENTS AND HEALTHY VOLUNTEERS

Variables	Original Cohort with Sepsis (*n* = 61)	Validation Cohort with Sepsis (*n* = 40)	Critically Ill without Sepsis (*n* = 44)	Healthy Volunteers (*n* = 32)
Metyrapone test				
8:00 A.M. 11β-deoxycortisol < 7 μg/dl	36 (59)*†	24 (60)*†	3 (7)	0 (0)
8:00 A.M. 11β-deoxycortisol < 7 μg/dl and ACTH < 150 pg/ml	29 (48)*†	16 (40)*†	1 (2)	0 (0)
Cosyntropin test				
Cortisol < 15 μg/dl	22 (36)*†	14 (35)*†	8 (18)*	27 (84)
Free cortisol < 2 μg/dl	32 (54)*	19 (50)*	16 (41)*	32 (100)
Δ Cortisol ⩽ 9 μg/dl nonresponders	17 (28)*	15 (38)*	15 (34)*	0 (0)

For definition of abbreviation, *see* Table 2.
Values are n (%).
* $p < 0.05$ for comparison with healthy volunteers.
† $p < 0.05$ for comparison with critically ill without sepsis.

respectively) than for cosyntropin-stimulated total and free cortisol levels (0.69 [95% CI, 0.59–0.75; p = 0.02] and 0.68 [95% CI, 0.54–0.68; p = 0.02]), and than for cosyntropin-stimulated increments in total and free cortisol levels (0.73 [95% CI, 0.60–0.84; p = 0.01] and 0.71 [95% CI, 0.59–0.88; p = 0.01]; Figure E2).

A baseline total cortisol level less than 10 μg/dl, or cosyntropin-stimulated increments in total cortisol less than 9 μg/dl and free cortisol less than 2 μg/dl, were strong predictors of adrenal insufficiency, having positive likelihood ratios equal to infinity, 8.46 (95% CI, 1.19–60.25), and 9.50 (95% CI, 1.05–9.54), respectively (Table E3). Furthermore, the combination "baseline total cortisol level less than 10 μg/dl or Δ total cortisol less than 9 μg/dl" was the stronger predictor of the presence of adrenal insufficiency. A cosyntropin-stimulated total cortisol level of 44 μg/dl or greater, and a cosyntropin-stimulated increment in total cortisol of 16.8 μg/dl or greater were predictive of the absence of adrenal insufficiency (Table 4). In the "validation cohort with sepsis," this combination correctly classified the adrenal function in 34/40 patients, with a sensitivity of 0.83 (95% CI, 0.74–0.95), a specificity of 0.88 (95% CI, 0.74–1.00), and a positive likelihood ratio of 6.67 (95% CI, 1.80–24.68) (Table 5 and Figure 3).

Finally, in a multiple logistic regression analysis, the strongest independent predictor of adrenal insufficiency was positive blood cultures, with an odds ratio of 10.2 (95% CI, 1.8–57.2).

DISCUSSION

In the present study, adrenal insufficiency was identified in 60% of patients with sepsis, and was associated with a greater likelihood

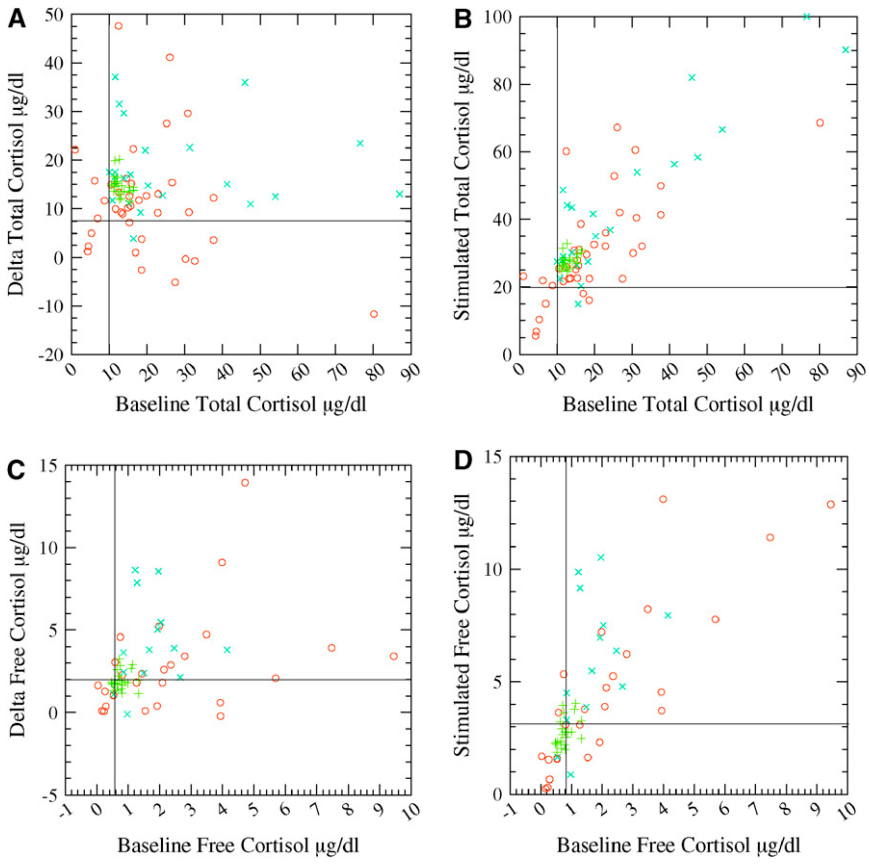

Figure 2. Plots of basal and absolute increment (delta) in total cortisol concentration (*A*), baseline and cosyntropin-stimulated total cortisol concentration (*B*), basal and absolute increment (delta) in free cortisol concentration (*C*), and baseline and cosyntropin-stimulated free cortisol concentration (*D*). *Vertical* and *horizontal straight lines* indicate cutoffs with higher specificity (i.e., 10 μg/dl for total cortisol concentration [*A* and *B*, *vertical line*], 9 μg/dl for delta of total concentration [*A*, *horizontal line*], 20 μg/dl for postcosyntropin total cortisol concentration [*B*, *horizontal line*], 0.8 μg/dl for basal free cortisol concentration (*C* and *D*, *vertical line*], and 2 μg/dl for delta of [*C*, *horizontal line*], and postcosyntropin free cortisol level [*D*, *horizontal line*]). *Open circles*, adrenal insufficiency; "×" *symbols*, normal adrenal function; "+" *symbols*, control subjects.

TABLE 4. ACCURACY OF VARIOUS COMBINATIONS OF CORTISOL AT BASELINE AND CORTISOL RESPONSE TO COSYNTROPIN

Diagnostic Tests	Sensitivity (*95% CI*)	Specificity (*95% CI*)	Positive Predictive Value (*95% CI*)	Negative Predictive Value (*95% CI*)	Positive Likelihood Ratio (*95% CI*)
Basal total cortisol concentration < 10 μg/dl or $\Delta \leq 9$ μg/dl	0.45 (0.26–0.63)	0.96 (0.87–1)	0.94 (0.86–1)	0.96 (0.89–1)	10.29 (1.47–72.27)
Basal total cortisol concentration < 10 μg/dl or cosyntropin-stimulated cortisol < 20 μg/dl	0.24 (0.08–0.39)	0.96 (0.87–1)	0.90 (0.78–0.99)	0.96 (0.88–1)	5.45 (0.74–40.25)
Basal free cortisol concentration < 0.8 μg/dl or $\Delta < 2$ μg/dl	0.57 (0.39–0.76)	0.84 (0.68–1)	0.87 (0.80–0.96)	0.90 (0.82–0.97)	3.62 (1.22–10.73)
Basal free cortisol concentration < 0.8 μg/dl or cosyntropin-stimulated cortisol < 3.1 μg/dl	0.46 (0.28–0.65)	0.90 (0.76–1)	0.84 (0.76–0.95)	0.84 (0.77–0.96)	4.41 (1.12–17.36)

Definition of abbreviation: CI = confidence interval.

of vasopressor dependency, severe cardiovascular dysfunction, and higher risk for in-hospital death. Using the overnight single-dose metyrapone stimulation test as a reference, the combination of "baseline cortisol level less than 10 μg/dl or a cosyntropin-stimulated total cortisol increment less than 9 μg/dl" was the best predictor of adrenal insufficiency. By contrast, the combination "cosyntropin-stimulated cortisol level of 44 μg/dl or greater, and an increment in total cortisol of 16.8 μg/dl or greater" excluded the diagnosis of adrenal insufficiency.

The observed prevalence of adrenal insufficiency in sepsis was higher than previously thought for critically ill patients (23). However, this was the first time that adrenal function in ICU patients was investigated using a test that assesses the whole hypothalamic–pituitary–adrenal axis. All previous studies were based on random cortisol levels or rapid corticotropin tests (2) that may underdiagnose adrenal failure in comparison with metyrapone testing (13). It is known that sepsis-induced cytokines may blunt the hypothalamic pituitary axis (17). In addition, recent data suggests that septic shock is associated with inducible nitric oxide synthase–induced neuronal apoptosis in the hypothalamus, which, in turn, may result in secondary adrenal failure (24). All healthy control subjects, and most of the ICU patients without sepsis, had normal metyrapone tests. In addition, patients with septic shock without adrenal insufficiency had responses to metyrapone that mimicked those of both healthy and critically ill control subjects. The time window of more than 8 h left between the ACTH and metyrapone tests allowed the avoidance of interference between the two tests, as previous studies have shown that cortisol levels returned to baseline values around 6 h after a 250-μg dose of ACTH (25). The overnight metyrapone test was feasible in almost all of the 112 screened patients with sepsis, with full absorption of the drug and sufficient inhibition of cortisol synthesis, excluding false-negative tests. All patients subsequently received corticosteroid replacement for at least 24 h, and tolerated the metyrapone test well.

In agreement with others (2), we found that fever, tachycardia, hypotension, multiple organ dysfunction, hyponatremia, hypoglycemia, or increased eosinophil count were inadequate to diagnose adrenal insufficiency. To the best of our knowledge, the strong association between bacteremia and the presence of adrenal insufficiency has not been previously reported. Until additional studies are available, clinicians should consider adrenal function testing in patients with bacteremia.

Previous studies proposing a baseline cortisol level less than 15 μg/dl as a diagnostic criterion for adrenal insufficiency in critically ill patients included a limited number of patients with sepsis (2, 26). In the present study, on a larger and more homogenous group of patients with sepsis, a total cortisol level less than 10 μg/dl more accurately predicted adrenal insufficiency, in keeping with findings obtained from a cohort of ICU patients with confirmed adrenal insufficiency (27). The present study confirms the diagnostic value of a Δ cortisol of less than 9 μg/dl after 250 μg cosyntropin stimulation (2, 6, 9). Baseline free cortisol level less than 0.8 μg/dl was more accurate in diagnosing adrenal insufficiency than the previously suggested 2-μg/dl value (7). This discrepancy between our study and that of Hamrahian (7) might be explained by the use of different populations (i.e., sepsis upon ICU admission versus a heterogeneous population with prolonged critical illness), or by difference in the determination of free cortisol level (calculated vs. measured). Nevertheless, free cortisol levels obtained in healthy control subjects and patients from both studies were closely comparable. Because free cortisol and CBG cannot be routinely measured in a timely fashion, we recommend using total cortisol levels in patients with sepsis.

Although most of our patients had septic shock, our findings suggest that adrenal insufficiency might be underappreciated in patients with severe sepsis. At present, little data are available in the literature on the incidence of impaired adrenal function in patients without septic shock. Similar to patients with septic shock and adrenal insufficiency (10), a recent randomized study reported a positive response to prolonged glucocorticoid supplementation in patients with severe community-acquired pneumonia; however, adrenal function was not tested (28). Another recent, randomized, placebo-controlled, double-blind trial showed a high prevalence of adrenal failure, as defined by a Δ cortisol

TABLE 5. VALIDATION OF THE DEFINITION OF ADRENAL INSUFFICIENCY IN 40 PATIENTS WITH SEPTIC SHOCK

	Sensitivity (*95% CI*)	Specificity (*95% CI*)	Positive Predictive Value (*95% CI*)	Negative Predictive Value (*95% CI*)	Positive Likelihood Ratio (*95% CI*)	Adrenal Insufficiency	
						Yes	No
Basal total cortisol concentration < 10 μg/dl or $\Delta \leq 9$ μg/dl—YES						20	2
Basal total cortisol concentration < 10 μg/dl or $\Delta \leq 9$ μg/dl—NO						4	14
Diagnostic tests							
Basal total cortisol concentration < 10 μg/dl or $\Delta \leq 9$ μg/dl—YES	0.83 (0.74–0.95)	0.88 (0.74–1)	0.9 (0.80–1)	0.88 (0.81–0.99)	6.67 (1.80–24.68)		

Definition of abbreviation: CI = confidence interval.

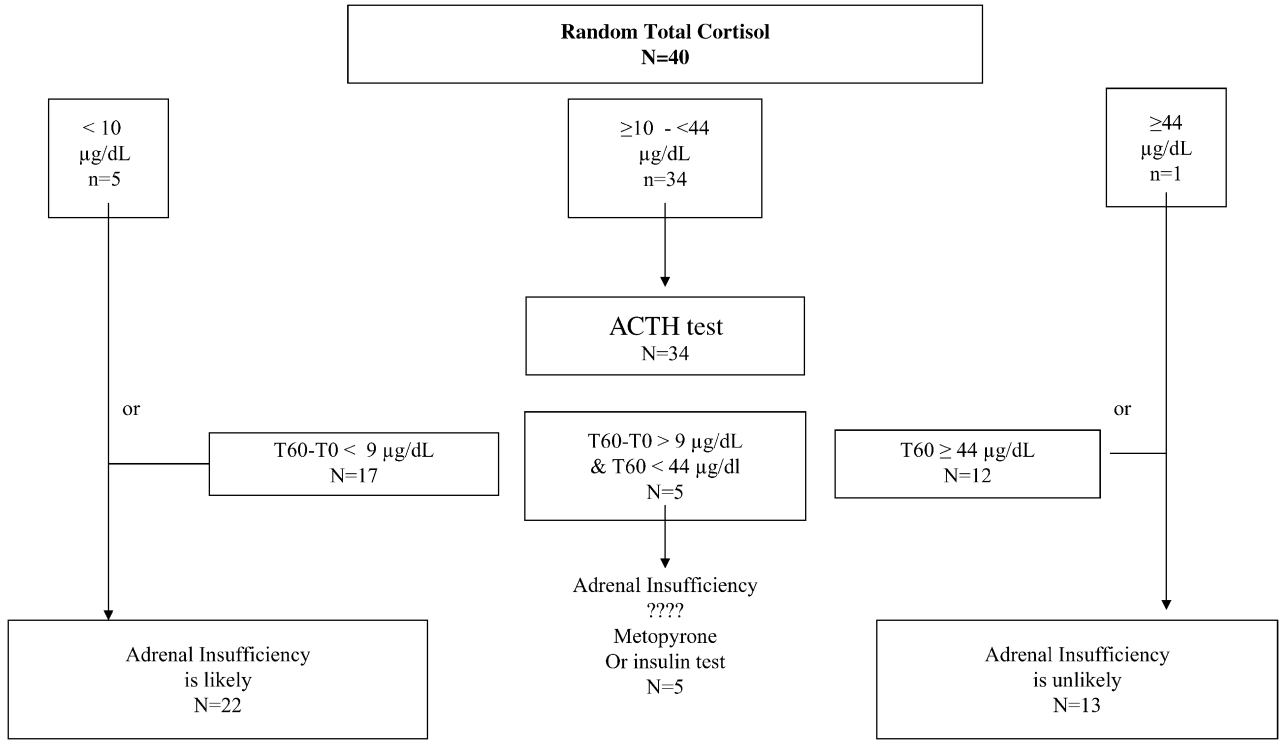

Figure 3. Distribution of the 40 patients from the validation cohort with sepsis along a decision tree for adrenal insufficiency.

of less than 9 µg/dl in patients who failed to be weaned from mechanical ventilation (29). Furthermore, when these patients were treated with replacement doses of hydrocortisone, they recovered to a probability of mechanical ventilation withdrawal similar to that of patients with presumed normal adrenal function.

In summary, physicians should systematically search for adrenal insufficiency in severe sepsis or septic shock, especially when blood cultures are positive. Patients with a baseline total cortisol level less than 10 µg/dl, or a cortisol increment after cosyntropin less than 9 µg/dl, are very likely to have adrenal insufficiency. Conversely, in patients with a cosyntropin-stimulated total cortisol level of 44 µg/dl or greater, or a cortisol increment after cosyntropin stimulation of 16.8 µg/dl or greater, adrenal insufficiency can be ruled out. When the baseline cortisol level is between 10 and 44 µg/dl, and the cortisol increment after cosyntropin stimulation is between 9 and 16.8 µg/dl, assessment of adrenal function requires metyrapone testing.

Conflict of Interest Statement: None of the authors has a financial relationship with a commercial entity that has an interest in the subject of this manuscript.

References

1. Waterhouse R. Case of suprarenal apoplexy. *Lancet* 1911;1:577.
2. Cooper MS, Stewart PM. Corticosteroid insufficiency in acutely ill patients. *N Engl J Med* 2003;348:727–734.
3. Oelkers W. Adrenal insufficiency. *N Engl J Med* 1996;335:1206–1212.
4. Arlt W, Allolio B. Adrenal insufficiency. *Lancet* 2003;361:1881–1893.
5. Marik PE, Zaloga GP. Adrenal insufficiency during septic shock. *Crit Care Med* 2003;31:141–145.
6. Rothwell PM, Udwadia ZF, Lawler PG. Cortisol response to corticotropin and survival in septic shock. *Lancet* 1991;337:582–583.
7. Hamrahian AH, Oseni TS, Arafah BM. Measurements of serum free cortisol in critically ill patients. *N Engl J Med* 2004;350:1629–1638.
8. Annane D, Bellissant E, Sébille V, Lesieur O, Mathieu B, Raphael JC, Gajdos P. Impaired pressor sensitivity to noradrenaline in septic shock patients with and without impaired adrenal function reserve. *Br J Clin Pharmacol* 1998;46:589–597.
9. Annane D, Sébille V, Troché G, Raphaël JC, Gajdos P, Bellissant E. A 3-level prognostic classification in septic shock based on cortisol levels and cortisol response to corticotropin. *JAMA* 2000;283:1038–1045.
10. Annane D, Sebille V, Charpentier C, Bollaert PE, Francois B, Korach JM, Capellier G, Cohen Y, Azoulay E, Troche G, *et al.* Effect of treatment with low doses of hydrocortisone and fludrocortisone on mortality in patients with septic shock. *JAMA* 2002;288:862–871.
11. Annane D, Bellissant E, Bollaert PE, Briegel J, Keh D, Kupfer Y. Corticosteroids for severe sepsis and septic shock: a systematic review and meta-analysis. *BMJ* 2004;329:480.
12. Azziz R, Fox LM, Zacur HA, Parker CR Jr, Boots LR. Adrenocortical secretion of dehydroepiandrosterone in healthy women: highly variable response to adrenocorticotropin. *J Clin Endocrinol Metab* 2001; 86:2513–2517.
13. Streeten DHP, Anderson GH Jr, Bonaventura MM. The potential for serious consequences from misinterpreting normal responses to the rapid adrenocorticotropin test. *J Clin Endocrinol Metab* 1996;81:285–290.
14. van den Berghe G, Wouters P, Weekers F, Verwaest C, Bruyninckx F, Schetz M, Vlasselaers D, Ferdinande P, Lauwers P, Bouillon R. Intensive insulin therapy in the critically ill patients. *N Engl J Med* 2001;345: 1359–1367.
15. Van den Berghe G, Wilmer A, Hermans G, Meersseman W, Wouters PJ, Milants I, Van Wijngaerden E, Bobbaers H, Bouillon R. Intensive insulin therapy in the medical ICU. *N Engl J Med* 2006;354:449–461.
16. American College of Chest Physicians/Society of Critical Care Medicine. Consensus conference: definitions for sepsis and organ failure and guidelines for the use of innovative therapies in sepsis. *Crit Care Med* 1992;20:864–874.
17. Prigent H, Maxime V, Annane D. Science review: mechanisms of impaired adrenal function in sepsis and molecular actions of glucocorticoids. *Crit Care* 2004;8:243–252.
18. McCabe WA, Jackson GG. Gram negative bacteremia: I. Etiology and ecology. *Arch Intern Med* 1962;110:847–855.
19. Le Gall JR, Lemeshow S, Saulnier F. A new simplified acute physiology score (SAPSII) based on a European/North American multicenter study. *JAMA* 1993;270:2957–2963.

20. Vincent JL, Moreno R, Takala J, Willatts S, De Mendonca A, Bruining H, Reinhart CK, Suter PM, Thijs LG, on behalf of the Working Group on Sepsis-Related Problems of the European Society of Intensive Care Medicine. The SOFA (Sepsis-related Organ Failure Assessment) score to describe organ dysfunction/failure. *Intensive Care Med* 1996;22:707–710.
21. Fiet J, Giton F, Boudou P, Villette JM, Soliman H, Morineau G, Boudi A, Galons H. A new specific and sensitive time resolved-fluoroimmunoassay of 11-deoxycortisol in serum. *J Steroid Biochem Mol Biol* 2001;77:143–150.
22. Coolens JL, Van Baelen H, Heyns W. Clinical use of unbound plasma cortisol as calculated from total cortisol and corticosteroid-binding globulin. *J Steroid Biochem* 1987;26:197–202.
23. Lamberts SWJ, Bruining HA, de Jong FH. Corticosteroid therapy in severe illness. *N Engl J Med* 1997;337:1285–1292.
24- Sharshar T, Gray F, Lorin de la Grandmaison G, Hopkinson NS, Ross E, Dorandeu A, Orlikowski D, Raphael JC, Gajdos P, Annane D. Apoptosis of neurons in cardiovascular autonomic centres triggered by inducible nitric oxide synthase after death from septic shock. *Lancet* 2003;362:1799–1805.
25. Siraux V, De Backer D, Yalavatti G, Melot C, Gervy C, Mockel J, Vincent JL. Relative adrenal insufficiency in patients with septic shock: comparison of low-dose and conventional corticotropin tests. *Crit Care Med* 2005;33:2479–2486.
26. Jacobs HS, Nabarro JD. Plasma 11-hydroxycorticosteroid and growth hormone levels in acute medical illnesses. *BMJ* 1969;2:595–598.
27. Bouachour G, Tirot P, Varache N, Gouello JP, Harry P, Alquier P. Hemodynamic changes in acute adrenal insufficiency. *Intensive Care Med* 1994;20:138–141.
28. Confalonieri M, Urbino R, Potena A, Piattella M, Parigi P, Puccio G, Della Porta R, Giorgio C, Blasi F, Umberger R, *et al.* Hydrocortisone infusion in patients with severe community-acquired pneumonia: a preliminary randomized study. *Am J Respir Crit Care Med* 2005;171:242–248.
29. Huang C-J, Lin H-C. Association between adrenal insufficiency and ventilator weaning. *Am J Respir Crit Care Med* 2006;173:276–280.