Free Cortisol and Accuracy of Total Cortisol Measurements in the Diagnosis of Adrenal Insufficiency in Brain-dead Patients

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ABSTRACT

Background: After brain death, adrenal insufficiency (AI) is very common and may be one of the mechanisms that contributes to hemodynamic instability and loss of potential organ donors. However, when diagnosed by total cortisol measurement, critically ill patients may be overdiagnosed as having AI. The aims of this study were to assess the prevalence of AI when diagnosed using free cortisol measurement and the accuracy of total cortisol measurement to diagnose AI in brain-dead patients.

Methods: All consecutive brain-dead patients were included in this single-center noninterventional clinical observation study. Assessment of adrenocorticotropin, corticosteroid-binding globulin, baseline and tetracosactin-stimulated serum free and total cortisol concentrations were performed. AI was defined as a baseline free cortisol concentration ≤ 55 nM^{-1} and/or Δ free cortisol ≤ 55 nM^{-1}. Patients were considered to have a low albumin concentration if less than 25 g·L^{-1} and a low corticosteroid-binding globulin concentration if less than 27 mg·L^{-1} in men or 31 mg·L^{-1} in women.

Results: Among the 42 included patients, the incidence of AI was 83% (95% CI, 69–93%). Baseline total cortisol was correlated with baseline free cortisol, whatever the albumin or corticosteroid-binding globulin concentration. The area under the receiver operating characteristic curve of baseline total cortisol measurement to diagnose AI was 0.94 (95% CI, 0.81–0.98). The optimal cutoff was 485 nM^{-1}, providing a sensitivity and a specificity of 89% and 100%, respectively.

Conclusion: Total baseline cortisol measurement is accurate and sufficient to diagnose AI in brain-dead patients, even if albumin or corticosteroid-binding globulin concentrations are low.

What We Already Know about This Topic

- Adrenal insufficiency is common in brain-dead patients and contributes to hemodynamic instability and loss of potential organ donation
- Whether total cortisol measurement is accurate to diagnose adrenal insufficiency in these patients, who frequently have low albumin and corticosteroid-binding globulin, is not known

What This Article Tells Us That Is New

- In 42 patients with brain death, total cortisol correlated strongly with free cortisol and accurately diagnosed adrenal insufficiency in the face of low circulating binding proteins

DESPITE improved management of patients on the transplant waiting list, the mortality during this period in the United States, as in other countries, has remained as high as 15% in 2005, with 42% of patients waiting for more than 2 yr.[1] Thus, increasing the number of potential donors is a real challenge.

Hemodynamic instability occurs in 80% of the potential donors and is at the origin of 10% of lost potential donors because of refractory hypotension despite adapted fluid infusion and norepinephrine administration.[2] During brain death, disruption of the hypothalamic pituitary-adrenal axis may be one of the mechanisms that contribute to this hemodynamic instability. Actually, despite lack of evidence of effect on outcome and transplant organ viability and function, high-dose corticosteroid replacement therapy is recommended in case of hemodynamic instability in potential organ donors.[3] We have previously shown that adrenocorticotropic hormone (ACTH) stimulation followed 1 h later by a supplementary dose of hydrocortisone enhanced the sys-

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Received from the Department of Anesthesiology and Critical Care and the Department of Emergency Medicine and Surgery, Université Pierre et Marie Curie-Paris 6, Groupe Hospitalier Pitié-Salpêtrière, Assistance Publique-Hôpitaux de Paris, Paris, France. Submitted for publication February 17, 2011. Accepted for publication June 6, 2011. Support was provided solely from institutional and/or departmental sources.

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temic hemodynamic stability in brain-dead patients.[4] Nevertheless, corticosteroid function in brain-dead patients is still a matter of debate, being found as normal,[5] decreased,[6] or increased.[7] Then, before studying the effect of low or high dose of corticosteroid therapy on transplant outcome, it seems necessary to better characterize adrenal insufficiency (AI) in brain-dead patients. Most often, AI is diagnosed by measurement of serum total cortisol, meaning serum-free cortisol plus the binding-globulin fraction of cortisol. However, only free cortisol is responsible for the physiologic action of the hormone. Because more than 90% of circulating cortisol is protein-bound (corticosteroid-binding globulin [CBG] and albumin), it is reasonable to suggest that alterations in the serum binding protein concentrations could affect measured serum total cortisol concentrations,[8] and thus the interpretation of tests used to assess adrenal function. Moreover, a misdiagnosis of AI may have been made in some critically ill patients because of a low baseline and tetracosactin-stimulated total cortisol related to hypoalbuminemia, despite normal circulating free cortisol concentrations.[8] However, the pathophysiologic basis of AI may markedly differ between living critically ill patients and brain-dead patients who may suffer from both hypothalamic-pituitary ischemia and relative adrenal gland insufficiency, and thus these previous results[8] may not apply to brain-dead patients.

The aims of this study were to assess the exact prevalence of AI when diagnosed with free cortisol measurements and to assess the ability of serum total cortisol concentration measurement to diagnose AI in brain-dead patients.

Materials and Methods

Patient Selection

All brain-dead patients 18 yr or older admitted to our intensive care unit for a potential multiple organ harvesting during a 12-month period were included prospectively. Before inclusion, brain death was diagnosed clinically with usual criteria (unresponsiveness to noxious pain stimuli, abolition of brainstem reflexes and apnea in the absence of hypothermia, metabolic or electrolyte disturbances and depressant drugs) and then was confirmed with cerebral angiography or electroencephalography, as previously described.[3] The inclusion of the patients in the study was performed before any discussion with families about potential organ donation. Patients were excluded if they had a history of hypothalamic–pituitary or adrenal disease or if they received corticosteroids during the previous 7 days or etomidate during the previous 3 days.[9] The study was approved by our ethics committee (Comité de Protection des Personnes de l'Hôpital Pitié-Salpêtrière, Paris, France) and was performed in accordance with the Declaration of Helsinki. This study was also conducted in accordance with French laws concerning multiple organ procurement. Waived informed consent was authorized because serum albumin measurement, diagnosis of adrenal insufficiency through serum total cortisol measurement, and hormone resuscitation are routinely performed in our unit for the assessment of brain-dead patients and because interventional procedures to evaluate the potential organ donor are authorized before the presumed consent could be verified. Free cortisol, ACTH, and CBG concentrations measurements were performed on blood withdrawn during routine biologic testing.

Study Protocol

Serum ACTH and albumin were measured and the capacity of adrenal gland to respond to tetracosactin was tested. Tetracosactin contains the first 24 amino acids of the natural ACTH sequence and has the same physiologic properties, similar to cosyntropin. We use the standard short tetracosactin stimulation, which measured free cortisol and total cortisol concentrations before (t0, baseline cortisol concentration) and 30 min (t30, stimulated cortisol concentration) after an intravenous injection of 250 μg tetracosactin (Synacthène®; Novartis Pharma SAS, Rueil Malmaison, France). Tetracosactin-stimulated absolute increment was defined as Δ cortisol = tetracosactin-stimulated cortisol concentration − baseline cortisol concentration. Serum samples were centrifugated and stored at −80°C for later analysis.

We defined adrenal insufficiency as baseline free cortisol concentration less than 55 nM^{-1} and/or Δ free cortisol less than 55 nM^{-1} in response to 250 μg tetracosactin, measured at t30.[8,10] A free cortisol concentration more than 55 nM^{-1} defined "ACTH responder" patients, whereas a free cortisol concentration less than 55 nM^{-1} defined "ACTH nonresponder" patients.[11] Patients were divided into two groups according to their serum albumin or CBG concentration. Hypoalbuminemia was defined as a serum albumin concentration lower than or equal to 25 $g{\cdot}L^{-1}$ and a low concentration of CBG was defined as lower than 27 $mg{\cdot}L^{-1}$ in men and lower than 31 $mg{\cdot}L^{-1}$ in women.[12]

Laboratory Analysis

Baseline plasma ACTH concentration was measured with the use of immunoradiometric assay kits (Immulite 2000, Siemens, Erlangen, Germany). When samples are withdrawn at 08:00 AM, values are considered as normal between 10 and 50 $pg{\cdot}mL^{-1}$. The range of "normal" values in critically ill patients is unknown. The intraassay and interassay coefficients of variation were less than 9% and less than 10% respectively. Measurements of serum total cortisol were performed with the use of a standard radioimmunoassay. The intraassay and interassay coefficients of variation were less than 2% and less than 3%. When samples are withdrawn at 08:00 AM, values are considered as normal between 170 and 535 nM^{-1}. The range of "normal" values in critically ill patients is unknown. Serum free cortisol concentrations were measured with the use of equilibrium dialysis of undiluted serum samples followed by radioimmunoassay. The intraassay and interassay coefficients of variation were less than 10% and less than 12%, respectively. Values are considered as normal between 2 and 43 nM^{-1} in males and nonestrogen-

treated females. The serum CBG concentration was measured by the Biosource radioimmunoassay (RIA; Lifescreen, Watford, Herts, United Kingdom) (normal range: males 27.1–52.3 mg·L^{-1}, females 31.0–53.4 mg·L^{-1}, and estrogen-treated females 64.4–116.0 mg·L^{-1}). Intraassay and interassay coefficients of variation were less than 6% and less than 9%, respectively. Measurements of serum free cortisol and CBG concentrations were performed at the Laboratoire de Radioanalyse, Institut de Physique Biologique, Faculté de Médecine, Strasbourg, France.

Statistical Analysis

There was no *a priori* sample size calculation: we based our study on the available patients during the 12-month period of the study. Data are expressed as mean ± SD for parametric variables or median (25–75 interquartiles) for nonparametric variables, or number and percentage and its 95% CI. Comparison of means was performed using the Student *t* test for parametric variables and comparison of medians using the Mann–Whitney U test for nonparametric variables. Proportions were compared using the Fisher exact test method. Correlations between two variables were calculated using Spearman rank test method. The area under the receiver operating characteristic curve was used to summarize the accuracy of serum baseline, stimulated and absolute increment after tetracosactin stimulation testing total cortisol in diagnosing AI. Optimal cutoffs for each measurement were determined using the Youden index (sensitivity + specificity − 1) to maximize appropriate classification.[13] All *P* values were two-tailed and a *P* value less than 0.05 was considered significant. Statistical analysis was performed using NCSS 2007 software (Statistical Solutions Ltd., Cork, Ireland).

Results

Forty-five consecutive brain-dead patients were screened in this prospective study. Three patients were treated with corticosteroids during the previous 7 days; none had received etomidate during the previous 3 days. Therefore, 42 patients, 18 women (43%) and 24 men (57%), 42 ± 17 yr old, were included in the study. The cause of brain death was trauma in 21 (50%) patients, cerebrovascular disease in 19 (45%) and anoxia in 2 (5%). The interval between accident and brain death was 85 ± 128 h.

The global incidence of AI defined by free cortisol was 83% (95% CI, 69–93%). Patients' patterns in regard to baseline free cortisol and responses to ACTH stimulation test are shown in figure 1. An ACTH concentration less than 10 pg·mL^{-1} was diagnosed in 25 patients (60%) and was not present in 17 (40%). Thirty-four patients (81%) were ACTH responders, whereas 8 (19%) were ACTH nonresponders (fig. 2). The incidence of no response to tetracosactin-stimulation test was similar in patients with low or normal baseline ACTH concentration (29% *vs.* 39%, respectively, $P = 0.50$). However, the interval between the causal

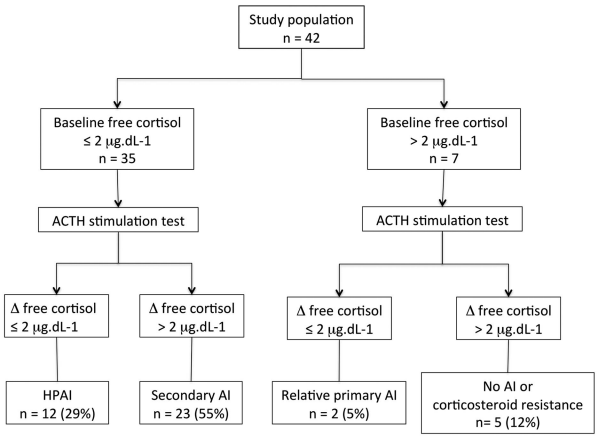

Fig. 1. Distribution of the patients according to baseline free cortisol and response to ACTH stimulation. ACTH = cortisol-adrenocorticotrophic hormone; Δ cortisol = δ free cortisol 30 min after 250 µg tetracosactin infusion; AI = adrenal insufficiency; HPAI = hypothalamic-pituitary-adrenal insufficiency.

accident and onset of brain death was shorter in ACTH nonresponders (37 ± 25 *vs.* 109 ± 152 h, $P = 0.02$).

Twenty-five patients (60%) had hypoalbuminemia and 17 (40%) did not. A slight but significant correlation between the interval between accident and brain death and albumin concentration was noted ($r^2 = 0.14$, $P = 0.01$). There was no significant difference in the incidence of AI between patients with low and normal albumin (80 *vs.* 88%, $P = 0.68$). Sixteen patients (38%) had a low CBG concentration and 26 (62%) did not. There was no significant difference in incidence of AI among patients with low and normal CBG concentration (88% *vs.* 81%, $P = 0.69$). Cortisol concentrations, with related albumin and CBG concentrations, are shown in table 1.

Baseline serum total cortisol concentrations were correlated with serum free cortisol concentrations in all patients ($r^2 = 0.83$, $P < 0.001$). There was no influence of albumin concentration on correlation between total and free cortisol (fig. 2). Patients with AI had lower baseline total cortisol concentrations than patients without AI (239 [127–393] nM^{-1} *vs.* 968 [572–1,245] nM^{-1}, respectively, $P < 0.001$) and lower tetracosactin-stimulated total cortisol concentration (833 [621–1,090] nM^{-1} *vs.* 1393 [1,238–1,464] nM^{-1}, respectively, $P = 0.003$), but there was no significant difference in Δ total cortisol (588 [430–782] nM^{-1} *vs.* 394 [302–58] nM^{-1}, respectively, $P = 0.18$). Individual data are shown in figure 3.

Areas under the receiver operating characteristic curves to diagnose AI were 0.94 (95% CI, 0.81–0.98) for baseline total cortisol concentration measurement, 0.83 (95% CI, 0.62–0.93) for tetracosactin-stimulated total cortisol, and 0.67 (95% CI, 0.38–0.83) for Δ total cortisol (fig. 4). The optimal cutoffs determined by the Youden index were 485 nM^{-1} for baseline total cortisol, 1,073 nM^{-1} for tetracosactin-stimulated total cortisol, and 199 nM^{-1} for Δ total cortisol. Baseline total cortisol measurement and tetracosactin-

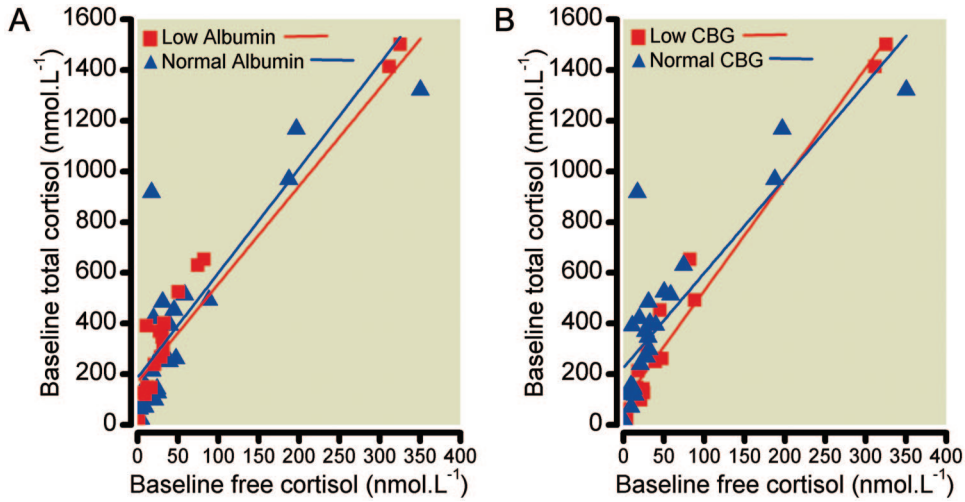

Fig. 2. (*A*) Correlation between baseline total and free cortisol concentrations in brain-dead patients with low (*dotted line*, n = 25, $r^2 = 0.73$, $P < 0.001$) or normal albumin (*solid line*, n = 17, $r^2 = 0.89$, $P < 0.01$) concentration. (*B*) Correlation between baseline total and free cortisol concentrations in brain-dead patients with low (*dotted line*, n = 16, $r^2 = 0.97$, $P < 0.001$) or normal corticosteroid-binding globulin (*solid line*, n = 26, $r^2 = 0.89$, $P < 0.01$) concentration. Alb = albumin; CBG = corticosteroid-binding globulin.

stimulated total cortisol measurement have similar sensitivities (89% *vs.* 74%, $P = 0.22$) and similar specificities (100% *vs.* 86%, $P = 1.0$). Combining test and Δ total cortisol did not increase the sensitivity or the specificity of the single baseline total cortisol test. The characteristics of each test are detailed in table 2.

Discussion

Our study confirmed the high prevalence of AI in brain-dead patients when diagnosed with a reference test, *i.e.*, free cortisol concentration measurement. However, given that this test is not routinely available we have demonstrated that, in brain-dead patients as opposed to living critically ill patients, the diagnosis of AI may be easily and accurately made with baseline total cortisol, whatever serum albumin or CBG concentrations. Moreover, adding a tetracosactin test and then increasing the cost of the diagnosis of AI did not increase the accuracy of the diagnosis.

Because cortisol is approximately 90% bound to CBG, 6% to albumin, and 4% is unbound or free and because only free circulating cortisol is biologically active,[14] it has been suggested that decrease in binding proteins could lead to misinterpretation of low serum total cortisol concentrations, thereby overestimating the incidence of AI.[8] In critically ill patients, these alterations in protein concentrations are very common because of hemodilution or loss of proteins (for example in patients with hypercatabolism, malnutrition, burns, hepatic failure, and nephrotic syndrome). In septic

Table 1. Serum Adrenocorticotropin Hormone (ACTH), Albumin, and Corticosteroid-binding Globulin (CBG) Concentrations and Baseline and Tetracosactin-stimulated Serum Total and Free Cortisol Concentrations in All Brain-dead Patients, in Patients with Low or Normal Albumin or Corticosteroid-binding Concentration

	All (n = 42)	Low Albumin (1) (n = 25)	Normal Albumin (2) (n = 17)	P (1) *vs.* (2)	Low CBG (3) (n = 16)	Normal CBG (4) (n = 26)	P (3) *vs.* (4)
ACTH ($pg \cdot mL^{-1}$)	8.5 (5.8–13.8)	—	—	—	6.4 (5.0–17.8)]	9.4 (5.0–17.8)	0.39
Albumin ($g \cdot L^{-1}$)	23.5 (19.3–27.8)	20.0 (15.5–21.8)	28.0 (27.0–32.0)	—	10.0 (17.0–25.0)	25 (22.0–29.0)	0.02
CBG ($mg \cdot L^{-1}$)	31.7 (25.2–37.3)	31.3 (21.8–37.3)	32.1 (28.5–37.1)	0.36	22.4 (18.1–25.3)	35.9 (32.1–40.7)	—
Total cortisol (nM^{-1})							
Baseline	29 (1,363–491)	251 (127–485)	346 (149–525)	0.38	232 (102–452)	358 (149–485)	0.41
Tetracosactin-stimulated	923 (632–1,258)	840 (635–1,107)	975 (629–1,475)	0.39	788 (519–840)	1,044 (891–1,385)	0.05
Δ Cortisol	578 (382–766)	571 (379–640)	584 (394–830)	0.55	468 (71–589)	630 (457–787)	0.03
Free cortisol (nM^{-1})							
Baseline	26 (]13–47)	24 (14–45)	29 (12–51)	0.87	28 (17–47)	25 (12–33)	0.40
Tetracosactin-stimulated	208 (110–334)	213 (112–292)	182 (110–370)	0.72	199 (70–248)	228 (149–359)	0.18
Δ Cortisol	146 (89–215)	158 (98–205)	134 (50–290)	0.87	102 (23–191)	165 (115–235)	0.03

Data are expressed as median (25–75 interquartiles).
ACTH = adrenocorticotrophic hormone; CBG = corticosteroid-binding globulin.

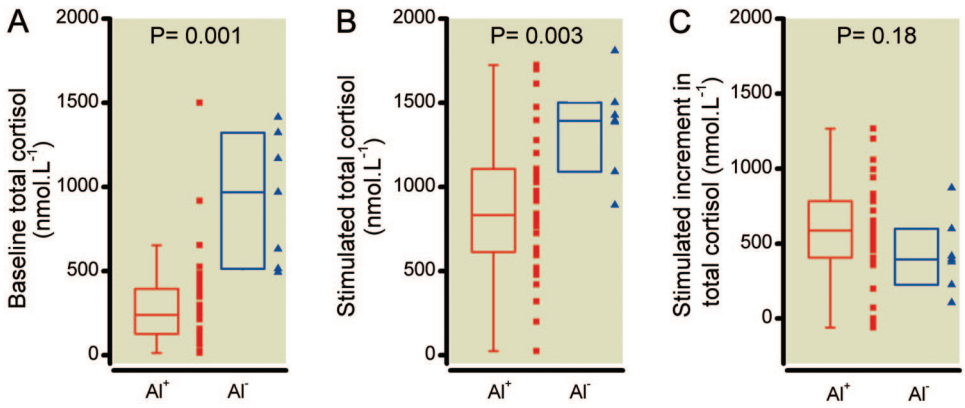

Fig. 3. Comparison of baseline (*A*), tetracosactin-stimulated (*B*), and Δ total cortisol (*C*) levels between patients with and without adrenal insufficiency (AI). Data individual and box plot (median, 25–75 percentiles, and whiskers).

shock, serum concentrations of CBG and albumin are decreased to approximately 50% of those observed in healthy control subjects.[15] In practice, total cortisol measurement has been found to have poor sensitivity and specificity and high variability in critically ill patients.[8,16]

In our study, more than one-half of brain-dead patients had hypoalbuminemia and 38% had a low CBG concentration. In contrast with a previous study of various living critically ill patients without cerebral injury,[8] we did not find any significant difference in CBG concentrations in brain-dead patients with or without hypoalbuminemia. CBG and albumin are saturated more quickly with cortisol when CBG and albumin concentrations decrease, especially in a stress situation when cortisol production is increased and the proportion of free cortisol increases. In his situation, Hamrahian *et al.*[8] have suggested that total cortisol measurement was not an effective way of diagnosing AI in critically ill patients. However, in our study, brain-dead patients with or without hypoalbuminemia had CBG concentrations similar to those in healthy volunteers (31.7 $mg{\cdot}L^{-1}$ and 26.8 $mg{\cdot}L^{-1}$, respectively), and higher than those in critically ill patients (17.7 $mg{\cdot}L^{-1}$ in hypoalbuminemia group and 21.4 $mg{\cdot}L^{-1}$ in normal albumin concentration group).[8] Thus, in brain-dead patients, because of persistence of unsaturated CBG and despite hypoalbuminemia, both bound and unbound cortisol may have increased when cortisol concentration is increased after stress. On the other hand, in the presence of AI, cortisol secretion is decreased and so both forms of cortisol, bound and unbound, may have decreased proportionally. This observation may explain the preserved correlation between total baseline and free cortisol concentrations, independently of albumin or CBG concentration, even if this result may be also underpowered due to modest sample size combined with the high influence of several extreme scores. Moreover, in terms of cortisol concentrations, AI was more severe in brain-dead patients than in living critically ill patients: despite stress, baseline free cortisol concentrations were similar to those of healthy volunteers and decreased by a factor of 6 to 7 compared with that of living critically ill patients.[8] This difference may be explained by the pathophysiologic basis of AI in brain-dead patients, which results from both an absolute AI due to brain death itself (hypothalamic-pituitary injury) and a relative AI due to incapacity of adrenal gland to response to the stress. Then, they may have a more severe AI than living critically ill patients. In practice, total cortisol measurement was not accurate enough to diagnose relative AI but accurate enough to diagnose severe AI, as in the case of brain-dead patients.

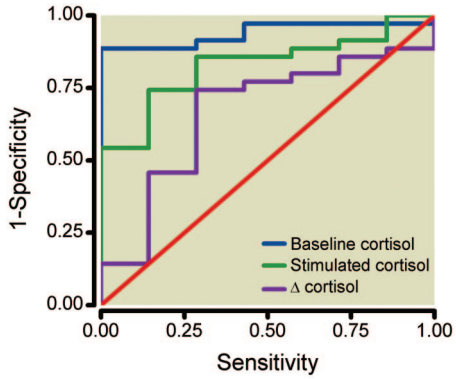

Fig. 4. Receiver operating characteristic curve showing the relationship between sensitivity and 1-specificity in determining the accuracy of baseline, tetracosactin-stimulated, and Δ total cortisol levels to diagnose adrenal insufficiency.

In our patients, baseline total cortisol ranged widely, from 14 to 1,546 nM^{-1}. This high interindividual variability has been reported in most previous studies.[7,17,18] In normal subjects, Weitzman *et al.*[19] have described the 24-h pattern of the episodic secretion of cortisol where peak total cortisol concentrations occur around the time of waking (10–20 $\mu g/dl^{-1}$, 276–552 nM^{-1}), whereas a minimum value (less than 5 $mg{\cdot}dL^{-1}$, <138 nM^{-1}) is observed at night. However, in the critically ill patients, circadian rhythm is not maintained. Moreover, in the case of stressed patients, there is no absolute cortisol concentration that distinguishes an adequate from an insufficient adrenal response. Some authors have suggested that a baseline total cortisol concentration lower than 15 $mg{\cdot}dL^{-1}$ (414 nM^{-1}) was inappropriate for a critically ill

Table 2. Diagnostic Value of Single Measurements of Baseline Total Cortisol, Total Tetracosactin-stimulated Cortisol, Tetracosactin-stimulated Absolute Increment in Total Cortisol, and Combined Measurements of Baseline Total Cortisol and Tetracosactin-stimulated Absolute Increment in Total Cortisol in Predicting an Adrenal Insufficiency in All Patients (n = 42)

Variables	Sensitivity	Specificity	PPV	NVP
TC_0 ≤485 nM^{-1}	0.89 (0.73–0.97)*	1.00 (0.59–1.00)	0.97 (0.85–0.99)	0.64 (0.31–0.89)*
TC_{30} ≤1,073 nM^{-1}	0.74 (0.57–0.88)*	0.86 (0.42–1.00)	0.96 (0.81–1.00)	0.40 (0.16–0.68)
ΔTC ≤199 nM^{-1}	0.14 (0.04–0.30)	0.86 (0.48–1.00)	0.83 (0.36–1.00)	0.17 (0.06–0.33)
TC_0 ≤485 or ΔTC ≤199 nM^{-1}	0.94 (0.81–0.99)*	0.71 (0.29–0.96)	0.94 (0.81–1.00)	0.71 (0.29–0.96)*

Data are expressed as percentage (95% CI). No significant difference between variables was observed between TC_0 ≤485 nM^{-1}, TC_{30} ≤1,073 nM^{-1} and TC_0 ≤485 nM^{-1} or ΔTC ≤199 nM^{-1}.

* $P < 0.05$ *vs.* TC_0 ≤485 nM^{-1} or ΔTC ≤199 nM^{-1}.

NPV = negative predictive value; PPV = positive predictive value; TC_0 = baseline total cortisol; TC_{30} = total tetracosactin-stimulated cortisol; ΔTC = tetracosactin-stimulated absolute increment in total cortisol.

situation.[20,21] Using receiver operating characteristic curves, we determined that the best cutoff of baseline total cortisol to diagnose AI in brain-dead patients was 485 nM^{-1}. Accordingly, at this cut-off, sensitivity and specificity of this assay were excellent, equal to 0.89 and 1.00, respectively. It should be noted that a short tetracosactin test was not useful for the diagnosis of AI because sensitivity of combined measurements was not higher than baseline total cortisol measurement alone (0.89 *vs.* 0.94, $P = 0.67$). We chose to use a high-dose tetracosactin stimulation (250 μg) instead of the low-dose stimulation (1 μg), even if the last one has been demonstrated to be more sensitive especially for the evaluation of secondary AI,[22] which seemed to be the major cause in our patients. However, because the low-dose tetracosactin stimulation is performed by diluting 1 ml tetracosactin in 249 ml normal saline and then administering 1 ml of the mixture, the preparation may be inaccurate. It is noteworthy that a recent meta-analysis had demonstrated that the low-dose stimulation is no more effective than the standard stimulation.[23]

Several limitations of our study should be noted. First, we did not find any significant influence of the albumin (or CBG) concentration on AI incidence, but the number of our brain-dead patients was small and the result may be due to a lack of power. To have reached such a power, we should have included a number as large as 402 brain-dead patients (201 with a normal albumin concentration and 201 with a low albumin concentration) to finally demonstrate a clinically irrelevant increase in AI incidence from 88%, as we have previously found,[4] to 95%. Second, in critically ill patients, the free cortisol cutoff level in defining AI is still a matter of debate. In these patients, because baseline free cortisol concentrations would be expected to exceed tetracosactin-stimulated concentrations in healthy unstressed subjects, Hamrahian *et al.*[8] recommended that a baseline serum free cortisol concentration of 55 nM^{-1} should be considered as a threshold that identifies patients at risk of AI. More recently, Annane *et al.*[10] considered metyrapone stimulation test as the reference test to diagnose AI and found a best cutoff of 22 nM^{-1} for baseline free cortisol and 55 nM^{-1} for Δ free cortisol. However, it should be noted that in that study, free cortisol was not measured but calculated, using the Coolens equation.[24] Because we did measure free cortisol, we choose to define AI by baseline cortisol levels as did Hamrahian *et al.*[8] Because 55 nM^{-1} was the same cutoff for Δ free cortisol calculated by Annane *et al.*,[10] we choose this cutoff to simplify the test. Nevertheless, we have shown that tetracosactin stimulation test was not necessary for the diagnosis. Currently, biologic cutoff levels remain a matter of debate, especially in critically ill patients.

In conclusion, our study demonstrates that total baseline cortisol measurement is accurate and sufficient to diagnose AI in brain-dead patients, even if low albumin or low CGB concentration occurs.

The authors thank the nursing team and the hospital coordinating the organ procurement team (Joelle Florit, nurse, Viviane Justice, nurse, Véronique Kokoszka-Biscompte, nurse, Isabelle Oilic, nurse) for their help with this study. We also thank David Baker, D.M., F.R.C.A. (Staff Anesthesiologist, Department of Anesthesiology, CHU Necker-Enfants Malades, Paris, France), for reviewing the manuscript.

References

1. Garrity ER, Moore J, Mulligan MS, Shearon TH, Zucker MJ, Murray S: Heart and lung transplantation in the United States, 1996–2005. Am J Transplant 2007; 7:1390–403
2. Salim A, Vassiliu P, Velmahos GC, Sava J, Murray JA, Belzberg H, Asensio JA, Demetriades D: The role of thyroid hormone administration in potential organ donors. Arch Surg 2001; 136:1377–80
3. Wood KE, Becker BN, McCartney JG, D'Alessandro AM, Coursin DB: Care of the potential organ donor. N Engl J Med 2004; 351:2730–9
4. Nicolas-Robin A, Barouk JD, Amour J, Coriat P, Riou B, Langeron O: Hydrocortisone supplementation enhances hemodynamic stability in brain-dead patients. ANESTHESIOLOGY 2010; 112:1204–10
5. Lopau K, Mark J, Schramm L, Heidbreder E, Wanner C: Hormonal changes in brain death and immune activation in the donor. Transpl Int 2000; 13 Suppl 1:S282–5
6. Howlett TA, Keogh AM, Perry L, Touzel R, Rees LH: Anterior and posterior pituitary function in brain-stem-dead donors: A possible role for hormonal replacement therapy. Transplantation 1989; 47:828–34
7. Powner DJ, Hendrich A, Lagler RG, Ng RH, Madden RL: Hormonal changes in brain dead patients. Crit Care Med 1990; 18:702–8

8. Hamrahian AH, Oseni TS, Arafah BM: Measurements of serum free cortisol in critically ill patients. N Engl J Med 2004; 350:1629–38
9. Vinclair M, Broux C, Faure P, Brun J, Genty C, Jacquot C, Chabre O, Payen JF: Duration of adrenal inhibition following a single dose of etomidate in critically ill patients. Intensive Care Med 2008; 34:714–9
10. Annane D, Maxime V, Ibrahim F, Alvarez JC, Abe E, Boudou P: Diagnosis of adrenal insufficiency in severe sepsis and septic shock. Am J Respir Crit Care Med 2006; 174:1319–26
11. Marik PE, Pastores SM, Annane D, Meduri GU, Sprung CL, Arlt W, Keh D, Briegel J, Beishuizen A, Dimopoulou I, Tsagarakis S, Singer M, Chrousos GP, Zaloga G, Bokhari F, Vogeser M, American College of Critical Care Medicine: Recommendations for the diagnosis and management of corticosteroid insufficiency in critically ill adult patients: Consensus statements from an international task force by the American College of Critical Care Medicine. Crit Care Med 2008; 36:1937–49
12. Dhillo WS, Kong WM, Le Roux CW, Alaghband-Zadeh J, Jones J, Carter G, Mendoza N, Meeran K, O'Shea D: Cortisol-binding globulin is important in the interpretation of dynamic tests of the hypothalamic–pituitary–adrenal axis. Eur J Endocrinol 2002; 146:231–5
13. Ray P, Le Manach Y, Riou B, Houle TT: Statistical evaluation of a biomarker. ANESTHESIOLOGY 2010; 112:1023–40
14. Arlt W, Stewart PM: Adrenal corticosteroid biosynthesis, metabolism, and action. Endocrinol Metab Clin North Am 2005; 34:293–313, viii
15. Ho JT, Al-Musalhi H, Chapman MJ, Quach T, Thomas PD, Bagley CJ, Lewis JG, Torpy DJ: Septic shock and sepsis: A comparison of total and free plasma cortisol levels. J Clin Endocrinol Metab 2006; 91:105–14
16. Clark PM, Neylon I, Raggatt PR, Sheppard MC, Stewart PM: Defining the normal cortisol response to the short Synacthen test: Implications for the investigation of hypothalamic-pituitary disorders. Clin Endocrinol 1998; 49:287–92
17. Gramm HJ, Meinhold H, Bickel U, Zimmermann J, von Hammerstein B, Keller F, Dennhardt R, Voigt K: Acute endocrine failure after brain death? Transplantation 1992; 54:851–7
18. Dimopoulou I, Tsagarakis S, Anthi A, Milou E, Ilias I, Stavrakaki K, Charalambidis C, Tzanela M, Orfanos S, Mandragos K, Thalassinos N, Roussos C: High prevalence of decreased cortisol reserve in brain-dead potential organ donors. Crit Care Med 2003; 31:1113–7
19. Weitzman ED, Fukushima D, Nogeire C, Roffwarg H, Gallagher TF, Hellman L: Twenty-four hour pattern of the episodic secretion of cortisol in normal subjects. J Clin Endocrinol Metab 1971; 33:14–22
20. 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; 283:1038–45
21. Cooper MS, Stewart PM: Corticosteroid insufficiency in acutely ill patients. N Engl J Med 2003; 348:727–34
22. Tordjman K, Jaffe A, Trostanetsky Y, Greenman Y, Limor R, Stern N: Low-dose (1 microgram) adrenocorticotrophin (ACTH) stimulation as a screening test for impaired hypothalamo-pituitary-adrenal axis function: Sensitivity, specificity and accuracy in comparison with the high-dose (250 microgram) test. Clin Endocrinol 2000; 52:633–40
23. Alia P, Villabona C, Gimenez O, Sospedra E, Soler J, Navarro MA: Profile, mean residence time of ACTH and cortisol responses after low and standard ACTH tests in healthy volunteers. Clin Endocrinol 2006; 65:346–51
24. 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