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Original Article

Determination of salivary cortisol to assess time-related changes of the adrenal response to stress in critically ill patients

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ABSTRACT

Background: The value of salivary cortisol measurement to study stress-related adrenal response is controversial. The study aim was to assess the role of salivary cortisol measurement to detect time-related changes of adrenal response in critically ill patients.

Patients and methods: Patients with organ failure, sepsis or trauma were prospectively recruited in the Emergency Department. Serum and salivary cortisol were measured at baseline (T0) and after 48 h (T48). In 33 patients ACTH test was also done.

Results: Fifty-five patients were studied and classified as septic (22) or non-septic (33). We found a significant correlation between serum and salivary cortisol at T0 and T48. No patient had baseline serum cortisol < 276 nmol/L and salivary cortisol significantly decreased at T48 in almost all patients. A delta serum cortisol < 250 nmol/L after ACTH was found in only 4 patients who showed elevated baseline cortisol levels.

Conclusion: We found that reduced baseline and post-ACTH cortisol levels are uncommon in our samples. In patients able to provide adequate saliva samples, salivary cortisol may be used to check the degree of stress-induced response and appears as a suitable tool for multiple measurements over time.

1. Introduction

The physiological response to acute stress and life-threatening conditions includes an increased activity of the adrenal glands resulting in raised cortisol levels, which are needed to support vital functions and restore homeostasis (fight or flight response) [1]. Studies pointed out that inadequate cortisol response to stressful stimuli might be associated with increased risk of mortality [2,3]. Therefore, the concept of "critical illness-related corticosteroid insufficiency" (CIRCI) has been introduced to define a condition characterized by baseline cortisol levels < 276 nmol/L or delta cortisol (difference between peak and baseline cortisol levels during a 250 μg ACTH stimulation test) < 250 nmol/L [4], although these criteria are still debated [5]. More recently, studies challenged this concept showing that high cortisol levels in critically ill patients were actually associated with increased risk of mortality [6–8]. In this area of uncertainty there is a further difficulty due to methodological issues related to cortisol measurement, because in acutely ill patients albumin and cortisol-binding protein (CBG) levels may be altered and a standardized method to evaluate free plasma cortisol is not currently available [9]. Although some evidence suggest that salivary cortisol could be a surrogate marker for serum free cortisol [10–14] data are lacking to support its routine use in critically ill patients [4].

Therefore, the aim of the present study was to determine cortisol levels in a population of critically ill patients in the first 48 h after their admission in the Emergency Room, and to test if salivary cortisol measurement may add useful information to serum cortisol measurement.

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2. Patients and methods

2.1. Patients

Patients were prospectively recruited in the Emergency Department of the San Giovanni Bosco Hospital (Turin), among those subsequently admitted in the Emergency Medical Unit, from September 2016 to December 2017. The study was conducted in accordance with the ethical guidelines of the 2003 (Declaration of Helsinki) and approved by the San Giovanni Bosco Hospital Ethics Committee. Written consent was obtained from patients and/or their designated family members.

Critically ill patients affected by a wide range of illness, and displaying single or multiple organ failure, were enrolled on the basis of the following inclusion and exclusion criteria. Inclusion criteria were: one or more of the followings: acute respiratory insufficiency, cardiovascular insufficiency or other organ insufficiency, sepsis or septic shock, acute pancreatitis, trauma (no brain injury), post-surgical complications. Diagnosis of sepsis and septic shock were based on the criteria established by the American College of Chest Physicians and the Society of Critical Care Medicine [15]. Exclusion criteria were: age < 18 years, ongoing mechanical ventilation, current or recent glucocorticoid therapy, neoplastic disease, intake of drugs known to influence glucocorticoids secretion (etomidate, ketoconazole, estrogen, phenytoin, phenobarbital and rifampicin), known pituitary, adrenal or liver disease, HIV or mycobacterial infection, pregnancy, immunosuppressive therapy. Patients were enrolled at the Emergency Room (ER) before admission. Assessment of the hypothalamic pituitary axis (HPA) was done at patient enrollment (T0) and after 48 h (T48), in concomitance with physical exam and routine biochemical evaluation. The severity of illness was assessed by the Simplified Acute Physiology Score (SAPS) II and qSOFA score [16]. The life status (death/alive) of the patients was checked at T48 and at day 28 after admission.

2.2. Methods

Within 24 h from admission, blood and saliva samples were concomitantly obtained from all patients (baseline, T0). Blood and saliva samples were also taken after 48 h (T48) from the first samples. Saliva samples were obtained by giving patients three cotton swabs to chew for 2 min. The cotton swabs were then placed in a saliva-collecting device (Salivette® – Sarstedt, Numbrecht, Germany) and centrifuged at 3000 rpm for 15 min. The cotton swabs were removed, and the samples were frozen at −20 °C until assayed. At baseline, salivary cortisol and serum cortisol were measured. At T48, salivary and serum cortisol were measured. Routine laboratory measurements included arterial blood gases, lactate, C-reactive protein, procalcitonin, blood electrolytes, total blood cells count, liver function tests, creatinine, and albumin.

Immediately after baseline sampling, 33 patients underwent a short corticotropin stimulation test. This test was performed between h.08.00 and 9.00 a.m. by intravenous administration of 250 μg of synthetic corticotropin (tetra-cosactide-hexa-acetate, Synacthen, Novartis Pharma, Basel, Switzerland). Blood and salivary samples were taken immediately before and 60 min after ACTH injection. The difference between serum cortisol at 60 min and at baseline (delta cortisol) was calculated to assess the response to ACTH stimulation. A subnormal response to ACTH stimulation was defined by a delta cortisol < 250 nmol/L [4].

2.3. Laboratory analyses

Salivary cortisol concentrations were determined using an ELISA kit (Enzyme-Linked ImmunoSorbent Assay), obtained from DGR instruments GmbH (Germany). The assay sensitivity was 1 nmol/L. As to specificity, this cortisol assay does not show cross-reaction with cortisone, deoxycorticosterone, 11-deoxycorticortisol, estradiol, testosterone, and 17-hydroxyprogesterone, while it cross-reacts 29% with corticosterone and 3% with cortisone. The reference range for our laboratory was 3–40 nmol/L, for both sexes.

Serum cortisol was measured by chemiluminescent immunometric assay (Immulite 1000, Siemens Medical Solutions Diagnostics, Los Angeles, CA, USA). The detection limit was 5 nmol/L. The reference range was 250–690 nmol/L.

2.4. Statistical analyses

All statistical analyses were performed with STATA statistical software. Categorical data were presented as rates and proportions, continuous data as medians and ranges. Wilcoxon rank-sum test was used for analysis of continuous variables and Chi-Square Test was used for nominal or ordinal explanatory and response variables. P values of < 0.05 were considered to indicate statistical significance.

3. Results

A total of 71 patients were eligible for inclusion in the study. One patient was excluded because he received a steroid pulse before admission and 15 patients were excluded because the amount of saliva samples was insufficient for the assay (Fig. 1).

Baseline characteristics of the 55 evaluated patients are reported in Table 1.

The patient group had different medical emergencies; the most frequent diagnosis at presentation was acute respiratory failure (50.9%) followed by acute heart failure (16.4%), suspected infections (16.4%), trauma (7.3%), acute pancreatitis (3.6%), septic shock (3.6%), or any organ failure (1.8%). A broad spectrum of comorbidities affected our patients, such as arterial hypertension (56.4%), diabetes mellitus (30.9%), coronary artery disease (25.4%), heart failure (21.8%), chronic kidney disease (20%), chronic obstructive pulmonary disease (20%), arrhythmia (14.5%), vascular disease (7.3%), liver disease (3.6%), pulmonary hypertension (3.6%), peripheral arterial disease (1.8%), peptic ulcer (1.8%), autoimmune diseases (1.8%). We divided our population in two subgroups based on the presence of sepsis, which was diagnosed in 22 out of 55 patients. Baseline characteristics of the two subgroups of patients are reported in Table 1.

In the overall sample, the median SAPS II score was 37 (16–77), without difference between the two groups (sepsis group, 36, 16–61 vs. no-sepsis group, 37, 17–77, $p = .76$). In the sepsis group, the median qSOFA was 1 [range 0–2].

Median serum cortisol concentration at T0 in the overall sample was 819 nmol/L (292–2458 nmol/L) and median salivary cortisol concentration at T0 was 41 nmol/L (range 1–347 nmol/L). No patient had baseline serum cortisol < 276 nmol/L. Median serum cortisol concentration at T48 was 634 nmol/L (287–1826 nmol/L) and median salivary cortisol concentration at T48 was 13 nmol/L (range 1–253 nmol/L). We found a significant correlation between serum and salivary cortisol concentrations measured at T0 (r 0.84, $p < .001$), and T48 (r 0.81, $p < .001$).

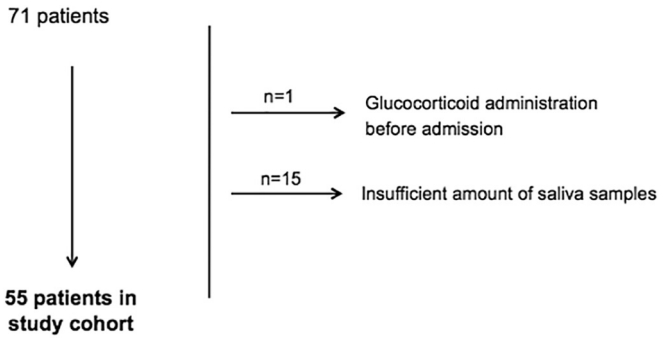

Fig. 1. Study cohort.

Table 1
Baseline features of the overall samples and the two groups classified according to presence of sepsis.

Characteristics	Overall (n = 55)	Sepsis (n = 22)	No Sepsis (n = 33)	*p*
Gender – N. (%)				
Male	36 (65%)	14 (63.6%)	22 (66.7%)	0.86
Female	19 (35%)	8 (36.4%)	11 (33.3%)	
Age at admission – yr				
Median [range]	70 [17-87]	65 [42-87]	71 [17-84]	0.49
BMI – kg/m^2				
Median [range]	26.8 [16.3-48.9]	26.0 [16.3-46.8]	27.5 [18.5-48.9]	0.10
Systolic Blood Pressure – (mmHg)				
Median [range]	130 [70-230]	125 [70-230]	136 [87-230]	0.07
Diastolic Blood Pressure – (mmHg)				
Median [range]	70 [35-130]	70 [40-130]	70 [35-120]	0.47
Heart rate – (bpm)				
Median [range]	90 [65-160]	99 [70-130]	86 [65-160]	0.32
Respiratory rate – (bpm)				
Median [range]	26 [12-46]	24 [12-46]	27 [14-44]	0.93
SO_2 – (%)				
Median [range]	94 [70-99]	96 [70-99]	94 [72-99]	0.91
Temperature – (°C)				
Median [range]	37 [36-41]	37.4 [36-41]	37 [36-39]	**0.01**
Lactate – (mmol/L)				
Median [range]	1.4 [0.1-8.3]	1.5 [0.5-7.7]	1.4 [0.1-8.3]	0.80
Protein - (g/dL)				
Median [range]	6.1 [4.3-7.9]	5.5 [4.3-7.8]	6.3 [5.5-7.1]	**0.02**
Albumin – (g/dL)				
Median [range]	3.1 [0.5-4.3]	2.8 [1.5-4.3]	3.4 [0.5-4.3]	**0.02**
Hospitalization – (day)				
Median [range]	11 [4-38]	11 [4-24]	10.5 [4-38]	0.73
Hospitalization in ED– (day)				
Median [range]	6 [1-21]	6 [2-15]	5 [1-10]	0.76

BMI = body mass index; SBP = systolic blood pressure; DBP = diastolic blood pressure; SO2 = hemoglobin oxygen saturation.
Bold indicates the *p* values with statistical significance ($p < 0.05$).

We found a statistically significant difference between salivary cortisol concentrations at T0 vs. T48 ($p < .001$) (Fig. 2).

In the sepsis group, salivary cortisol concentration decreased from 60 nmol/L (range 1–347 nmol/L) at T0 to 21 nmol/L (1–135 nmol/L) at T48 ($p < .001$). In the no-sepsis group, salivary cortisol concentration decreased from 39 nmol/L (range 1–221 nmol/L) at T0 to 11 nmol (range 1–253 nmol/L) at T48 ($p = .022$) (Fig. 3). We also compared salivary cortisol concentrations between the two groups and we found a statistically significant difference in salivary cortisol values at T48 ($p = .03$), while no significant difference was apparent at T0 (Fig. 3).

We did not find any significant correlation between serum or salivary cortisol and SAPS values.

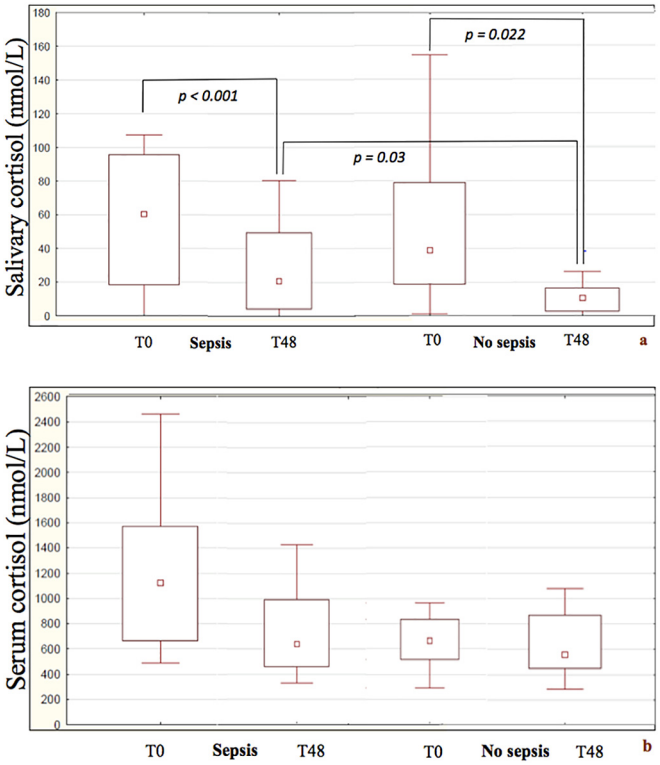

Fig. 3. Salivary (3a) and serum (3b) cortisol concentration at T0 and T48, in sepsis and no sepsis group.
The box identifies the 25–75% range and the whisker the non-outlier range.

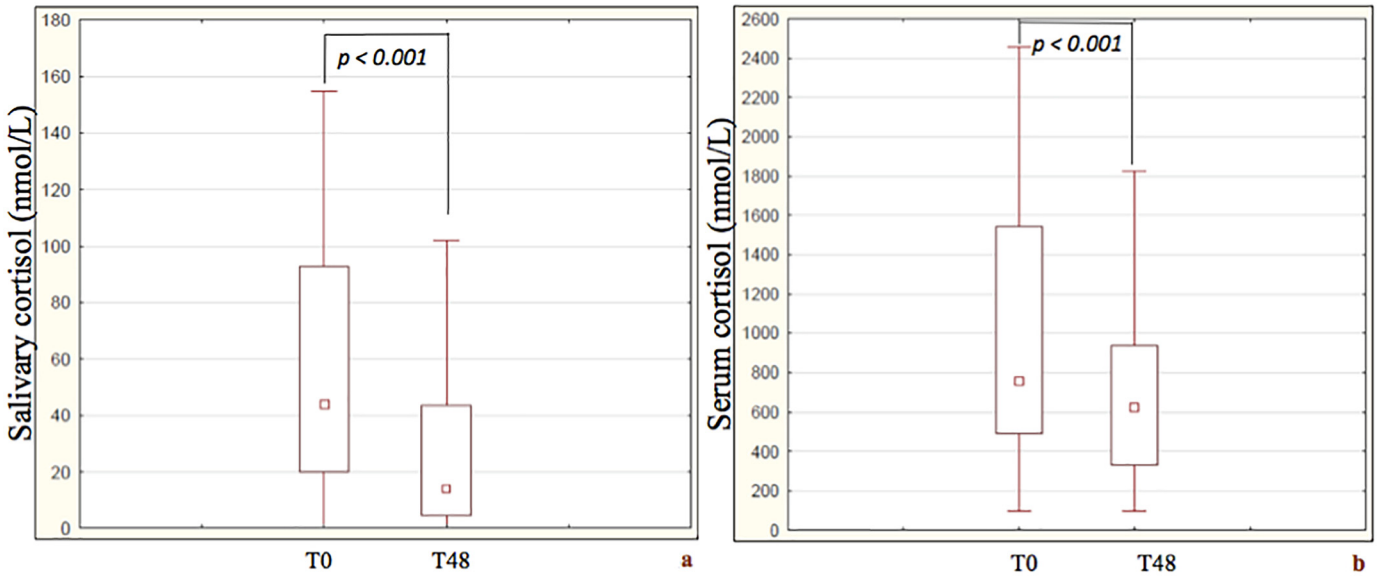

Fig. 2. Salivary (2a) and serum (2b) cortisol concentration in the overall sample at T0 and T48.
The box identifies 25–75% range and the whisker the non-outlier range.

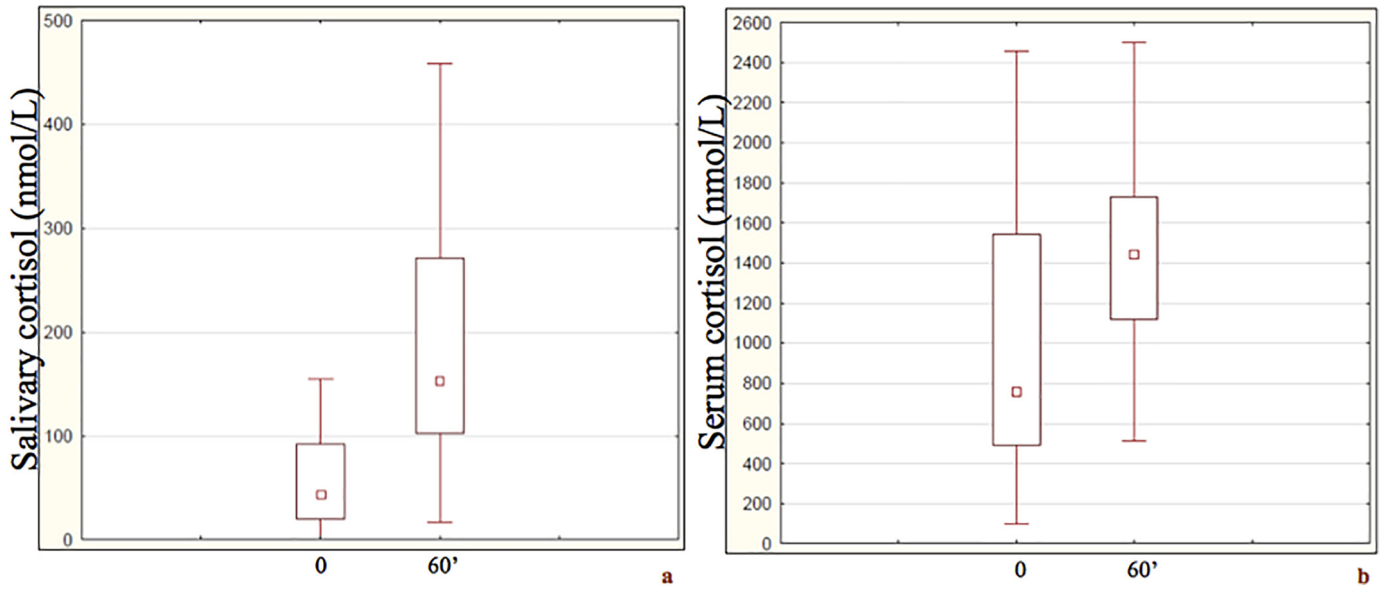

Fig. 4. Salivary (4a) and serum cortisol (4b) before and after ACTH stimulation test.
The box identifies the 25–75% range and the whisker the non-outlier range.

For logistic reasons (i.e. night-time admission), the ACTH stimulation test was performed in 33 patients. The serum cortisol value at 60 min was 1534 nmol/L (510–2676 nmol/L), and the salivary cortisol was 156 nmol/L (range 17–1528 nmol/L); with a significant correlation between them (r 0.49, $p < .01$). The delta serum cortisol was 588 nmol/L (range − 110–1432 nmol/L) and the percentage of the increase was 79% (range − 6–393%). The delta salivary cortisol was 113 nmol/L (range − 54–1308 nmol/L) and the percentage of the increase was 191% (range − 16 –1672%) (Fig. 4**).**

We found a delta serum cortisol < 250 nmol/L in 4 out 33 cases (12.1%). These patients had exceedingly high serum cortisol levels at T0 (1581; 1545; 2458; 1975 nmol/L, respectively), high values of SAPS (39, 58, 61, 77, respectively), increased lactate concentrations, and sepsis was diagnosed in three of them. The response of salivary cortisol to Synacthen was also blunted (percent of increment 37%, −7%, −16%, 18%, respectively).

Mortality rate was 1/55 patients at T48, and 5/55 patients at day 28. Due to the low mortality rate observed in our cohort, we did not analyze the correlation with cortisol levels.

4. Discussion

In the last 30 years, there was a fierce debate on the existence of a correlation between mortality and cortisol levels in critically ill patients, particularly when levels are at the lower end of the spectrum, configuring the so-called CIRCI [2,4,5], or when they are exceedingly high [6–8]. In both cases, the assessment of serum cortisol is not completely reliable, due to the decrease of CBG and albumin in critically ill patients [17]. As a matter of fact, 80–90% of serum cortisol is bound to CBG, 5–10% is bound to albumin, while only 5% is unbound [18]. At the same time, the laboratory evaluation of free serum cortisol is difficult, requiring special equipment. On the contrary, salivary cortisol measurement is cheap, noninvasive and unaffected by variations in CBG or albumin.

The recent guidelines on CIRCI diagnosis and management suggest against the use of salivary cortisol for diagnostic purposes, although this is a conditional recommendation with a very low quality of evidence [5]. One of the objections raised in the guidelines concerns the difficulty in collecting adequate salivary samples in critically ill patients, often intubated and dehydrated. Studies report that it was impossible to analyze about 30% of the salivary samples, due to insufficient saliva, or blood contamination [13,14]; conversely, in one study [12] only 5.5% of enrolled patients had inadequate saliva samples. In the present study, we confirm that collecting saliva is not an easy task in the ER, since 21.4% of our patients were excluded due to a small volume of saliva collected. This discrepancy among the studies is probably due to different types of population analyzed, with variable grade of illness severity and number of intubated patients. Whether the use of different saliva-collecting devices (for example infant-swab-devices) could ameliorate the success of obtaining adequate saliva samples in critically ill patients deserves future investigation.

Many studies supported the use of salivary cortisol as a surrogate for free cortisol in patients with critical illness, because it is as reliable as serum free cortisol, but not affected by the high cost and low availability of equilibrium dialysis, needed to determine free serum cortisol [12–14].

This concept was confirmed by our study, where we found a significant correlation between serum and salivary cortisol measurements. The strong correlation between salivary cortisol and total serum cortisol levels has been demonstrated not only at baseline, as in previous studies [12–14] but also after 48 h of hospitalization. This is a new element introduced by our research reporting a statistically significant difference between T0 and T48 salivary cortisol concentrations in the whole group, and in the two subgroups of septic and non-septic patients. We showed that salivary cortisol decreases after 48 h, a finding that likely reflects the shutting down of adrenal hyperactivity. Interestingly, the change in salivary cortisol seems to be more sensitive and precocious that serum cortisol, which was less modified over time. We observed a similar pattern during ACTH stimulation test. There are scanty data on the response of salivary cortisol to Synacthen and a universally accepted cut-off is not available [12,19,20], due to the great heterogeneity of the assays used to measure salivary cortisol between different studies.

This is the first study with a direct comparison between septic and non-septic patients, classified on the basis of the recent diagnostic criteria of sepsis [16]. In our study, we did not find any correlation between the severity of illness and salivary or serum cortisol levels. Therefore, our data do not seem to support the hypothesis that salivary cortisol increases in parallel to the severity of stress [13]. However, we may disclose the fact that the severity of illness and sepsis was moderate in many patients. Moreover, we found a significantly higher value of salivary cortisol at T48 in the group of septic patients, which may

reflect a more persistent activation of the HPA axis in this condition. We also found a correlation between serum and salivary cortisol at 60 min of the ACTH stimulation test. The role of salivary cortisol as surrogate for serum free cortisol in dynamic testing of adrenocortical function has been previously investigated [12,19,20], and our findings further support the use of salivary cortisol in this setting.

We did not find any evidence of CIRCI in our cohort, since no patient had baseline serum cortisol < 276 nmol/L, and a delta serum cortisol < 250 nmol/L after ACTH stimulation was observed in only 12% of cases. However, baseline cortisol levels were very high in such patients, suggesting that the reduced delta cortisol value may reflect a maximally stimulated HPA axis more than adrenal insufficiency. Interestingly, the 4 patients with a delta serum cortisol < 250 nmol/L after ACTH stimulation had concomitantly blunted salivary cortisol response.

We acknowledge the limitations of the study, including a small sample size and the heterogeneity of the population. Moreover, it could be argued that the low severity of illness in our patients, confirmed by a low mortality rate, is also a limitation.

To conclude, proven that patients are able to collaborate and provide adequate saliva samples, salivary cortisol may be used to check the degree of stress-induced activation of the HPA axis, and appears as a suitable tool for multiple measurements over time.

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Declaration of Competing Interest

The authors have stated explicitly that there are no conflicts of interest in connection with this article.

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