Cortisol responses at baseline and after corticotropin in acute aneurysmal subarachnoid haemorrhage: a prospective study

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Abbreviations

AI	adrenal insufficiency
aSAH	aneurysmal subarachnoid haemorrhage
CBG	corticosteroid binding globulin
CV	coefficient of variation
FPC	free plasma cortisol
HPAA	hypothalamic–pituitary–adrenal axis
SST	short Synacthen test
TPC	total plasma cortisol
WFNS	World Federation of Neurosurgical Societies

ABSTRACT

Background: Measurements of total plasma cortisol (TPC) in the acute phase of aneurysmal subarachnoid haemorrhage (aSAH) have suggested a high incidence of adrenal insufficiency (AI).

Objective: To compare TPC and free plasma cortisol (FPC) measurements in acute aSAH and to assess whether rates of diagnosis of AI based on TPC and FPC criteria were discordant.

Methods: A prospective, observational study of 20 patients admitted within 7 days of aSAH to a tertiary intensive care unit. Cortisol binding globulin (CBG), TPC and FPC levels were measured at baseline, and cortisol profiles at 30 and 60 minutes after administration of 250 μg corticotropin.

Results: Compared with controls, the mean baseline FPC (46 nmol/L [SD, 48 nmol/L] v 9 nmol/L [SD, 6 nmol/L], $P < 0.0001$), and TPC (566 nmol/L [SD, 288 nmol/L] v 352 nmol/L [SD, 146 nmol/L], $P = 0.01$) were significantly elevated with a greater proportional increase of FPC over TPC (6 v 1.2 times, $P < 0.0001$). The relative increment of FPC compared with TPC in the patient group was 505% v 114% ($P < 0.0001$) and in the control group was 662% v 145% ($P < 0.0001$). The prevalence of AI, measured using TPC compared with FPC, was 30% v 0% ($P = 0.04$).

Conclusion: In the acute phase after aSAH, the FPC increase is fivefold greater than that of TPC. There is discordance between TPC and FPC responses to corticotropin. The prevalence of AI, as assessed by FPC measurements, is negligible. We advocate caution in the assessment of adrenal cortical function using measurements of TPC in this population.

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Aneurysmal subarachnoid haemorrhage (aSAH) is a relatively uncommon but catastrophic condition. The incidence in Australia is 1 in 10000,[1] and the mortality rate is 39%.[2] Recent studies have documented abnormalities in the hypothalamic–pituitary–adrenal axis (HPAA) in the acute phase of aSAH. Using criteria derived from critically ill patients, the incidence of adrenal insufficiency (AI) in this population group has been reported as between 17% and 60%.[3,4] This finding has led to the suggestion that there may be a role for treatment with glucocorticoids in patients with aSAH and abnormal findings on testing of the HPAA.[5]

In critical illness, the recommended criteria for defining AI are based on measurement of total plasma cortisol (TPC) from a single measurement or in response to a short Synacthen test (SST) using corticotropin 250 μg.[6] However, the validity of measuring TPC in these circumstances is questionable, as it does not differentiate between protein-bound cortisol and the free fraction of cortisol.[7] About 95% of circulating cortisol is protein bound, primarily to corticosteroid binding globulin (CBG). This is the portion that exerts a biological effect, therefore CBG may be a more valid measure of adrenal function.[8] Free plasma cortisol (FPC) can be calculated using measurements of TPC and CBG concentrations using the Coolens equation, but we recently showed that this approach is inaccurate in the critically ill population.[9] Direct measurement is therefore the preferred approach but this has not been reported in patients with aSAH. Additionally, what constitutes a normal FPC response in critically ill patients remains uncertain, as several threshold values derived from different measurement techniques have been reported.

To further investigate the incidence of AI after aSAH, we performed a prospective observational study to compare:

- the TPC response with the FPC response, measured using ultra-high-performance liquid chromatography tandem mass spectrometry (which is the accepted gold standard)
- the prevalence of AI using TPC with the prevalence of AI using FPC, using normal values obtained from a healthy control population
- our measured values with those obtained by calculation from the Coolens equation.

Methods

Participants

This was a prospective study conducted at a tertiary referral hospital between November 2012 and August 2013. The study protocol was approved by the Royal Brisbane and Women's Hospital research ethics committee. Consent was given by all participants or their next of kin before enrolment in the study. All patients between 18 and 80 years of age presenting with a diagnosis of ruptured aSAH within the previous 7 days were screened for inclusion. Exclusion criteria included pregnancy, known primary AI, prolonged (> 2 weeks) courses of steroid treatment within the previous 6 months, and short-term (< 2 weeks) courses of steroid treatment within the previous 4 weeks. Patients were managed by each institution using their standard protocols on blood pressure control, early fixation of the aneurysm, fluid management and treatment of hydrocephalus. All patients received nimodipine and none received hydrocortisone or ketoconazole.

To allow comparisons with control subjects, data were included from healthy volunteers participating in a concurrent study (unpublished and undergoing data analysis) for which ethics approval had been received. Control subjects filled in a standard health questionnaire and were excluded from participation if they were pregnant or had a history of AI or steroid treatment as above.

Laboratory investigations

On the day of the study, blood samples were collected for baseline total and free cortisol levels and CBG measurements through indwelling arterial cannulae. An SST was then performed using corticotropin (Synacthen, Novartis) 250 μg intravenously, with further blood samples collected at 30 and 60 minutes after administration. The time of collection was not standardised, but occurred during working hours (8 am to 6 pm).

The control subjects underwent an SST between 8 am and 9 am in an outpatient clinic setting. The SST was performed by administering corticotropin 250 μg through a peripheral intravenous cannula. Serial TPC and FPC levels were measured via collection through the cannula, before corticotropin administration, and at 30 and 60 minutes after administration. Baseline blood samples were also analysed for CBG concentration.

Table 1. Patient demographics

Characteristic	Patients (n=20)	Controls (n=20)
Age (years)*	59 (28–77)	28 (20–46)
Women, *n* (%)	15 (75%)	8 (40%)
APACHE II score*	15 (5–28)	–
WFNS classification*	3 (1–5)	–
Days after aneurysm rupture*	3 (1–5)	–

APACHE II = Acute Physiology and Chronic Health Evaluation. WFNS = World Federation of Neurosurgeons. * Median and interquartile range.

Blood samples were collected into serum tubes (Becton, Dickinson) at room temperature, allowed to clot and then centrifuged at 2200 G for 10 minutes. Ultrafiltrates of serum for the free hormone assays were prepared by equilibrating 500 μL of plasma at 37°C for 15 minutes in Amicon Ultra-4 regenerated cellulose, 30000 molecular weight cut-off, centrifugal filter devices (Merck Millipore) before centrifugation at 3040 G for 20 minutes at 37°C. Aliquots of serum and ultrafiltrates were stored at –20°C until analysis in a single batch. TPC and FPC were measured using an ultra-high-performance liquid chromatography tandem mass spectrometry method, as described in detail by Pretorius and colleagues and McWhinney and colleagues.[10,11] An Acquity ultra-high-performance liquid chromatography system (Waters Corporation) was used, with a Micromass Quatro Premier XE mass spectrometer (Waters Corporation), to measure cortisol in the plasma ultrafiltrate. The limit for quantitation of TPC was 3.75 nmol/L (coefficient of variation [CV], 7.4%) and for FPC was 0.6 nmol/L (CV, 10%). The interassay CV (intermediate precision) for TPC was 4.6% at 77.5 nmol/L, and 3.1% at 729 nmol/L, and for FPC was 5.6% at 25 nmol/L and 3.1% at 76.9 nmol/L. CBG was measured by radioimmunoassay. The interassay CV quoted by the manufacturer (IBL International GMBH) for this assay is 6.2% at 30 mg/L and 5.1% at 111 mg/L.

The peak cortisol concentration was defined as the highest cortisol value at 30 or 60 minutes after corticotropin administration. The cortisol increment was defined as the difference between the peak and baseline cortisol levels, and the relative increment was defined as the peak/baseline cortisol concentration. AI was diagnosed by TPC and FPC criteria. TPC criteria were defined from published guidelines as a baseline value of < 276 nmol/L or an increment of ⩽250 nmol/L.[6] FPC criteria for AI, derived from published datasets in healthy volunteers, were defined as peak concentrations after corticotropin of < 33 nmol/L.[12–15]

Statistical analysis

We used GraphPad Prism, version 5 for Windows (GraphPad) for statistical analysis. Continuous variables are reported as means with standard deviations. Differences in cortisol concentrations between patient and control groups were analysed using the Mann-Whitney *U* non-parametric rank-sum test and unpaired *t* tests. The McNemar test was used to compare the prevalence of AI using TPC and FPC criteria. A P value < 0.05 was taken to be statistically significant. Due to the exploratory nature of the study and the lack of pre-existing data, formal power calculations were not performed.

Table 2. Cortisol measurements at baseline and after corticotropin 250µg

Cortisol measure	Patients (n = 20)	Controls (n = 20)	P
Mean total cortisol, nmol/L (SD)			
Baseline	566 (288)	352 (146)	0.01
Peak	1031 (278)	780 (197)	0.0007
Increment	466 (208)	428 (118)	NS
Mean free cortisol, nmol/L (SD)			
Baseline	46 (48)	9 (6)	<0.0001
Peak	152 (64)	56 (14)	<0.0001
Increment	106 (52)	47 (13)	<0.0001
Mean CBG, mg/L (SD)			
Baseline	33.5 (9)	60.2 (24)	0.0003

CBG = cortisol binding globulin. NS = not significant.

Results

Demographic data

Fifty-two patients were screened for eligibility and 20 patients were enrolled. Results from 20 controls were available. Demographic data are shown in Table 1. Reasons for non-eligibility included a non-aneurysmal bleed (10 patients), consent refusal (nine), a bleed >7 days beforehand (six) and other reasons (seven).

At the time of the study, 12 patients had their aneurysm secured with coiling (60%), six by clipping (30%), and the remaining left unsecured (10%). Seven patients needed mechanical ventilation (35%), four needed vasopressor support (20%), 11 were sedated (55%), and four were receiving fludrocortisone (20%). Six patients (30%) suffered one or more clinical episodes of vasospasm, defined as a new onset neurological deficit with evidence of arterial narrowing on cerebral imaging. No patients received etomidate or hydrocortisone during the study period.

Cortisol profiles

TPC and FPC profiles for patients and controls are shown in Table 2. The TPC at baseline was significantly elevated in aSAH patients compared with controls (566 nmol/L [SD, 288 nmol/L] v 352 nmol/L [SD, 146 nmol/L], P = 0.01). A similar pattern was observed for the free cortisol measurements (46 nmol/L [SD, 48 nmol/L] v 9 nmol/L [SD, 6 nmol/L], P < 0.0001) (Figure 1). The response to the SST was significantly greater in the FPC fraction in patients and controls; the relative increment of FPC compared with TPC in the patient group was 505% v 114% (P < 0.0001) and in the control group was 662% v 145% (P < 0.0001) (Figure 2). When comparing the cortisol increment in response to the SST, there was no difference between the aSAH group and controls in the total cortisol response (466 nmol/L [SD, 208 nmol/L] v 428 nmol/L [SD, 118 nmol/L], P = 0.4); whereas the free cortisol increment was significantly greater in the aSAH group (106 nmol/L [SD, 52 nmol/L] v 47 nmol/L [SD, 13 nmol/L], P < 0.0001).

CBG levels were significantly lower in aSAH patients compared with controls (33.5 mg/L [SD, 9 mg/L] v 60.2 mg/L [SD, 24 mg/L], P = 0.0003).

We found no significant difference between the TPC or FPC values at baseline in patients with or without vasospasm (TPC, 731 nmol/L [SD, 369 nmol/L] v 495 nmol/L [SD, 208 nmol/L]; FPC, 59 nmol/L [SD, 73 nmol/L] v 40 nmol/L [SD, 30 nmol/L]; P = not significant). Likewise, there was no observed difference in cortisol fractions between patients with low-grade (World Federation of Neurosurgical Societies [WFNS] grade 1–2) or high-grade (WFNS grade 3–5) injuries (TPC, 606 nmol/L [SD, 376 nmol/L] v 539 nmol/L [SD, 205 nmol/L]; FPC, 58 nmol/L [SD, 68 nmol/L] v 37 nmol/L [SD, 24 nmol/L]; P = not significant).

Adrenal insufficiency

Six patients (30%) were diagnosed with relative AI, using TPC criteria, having either a baseline TPC <276nmol/L, or a delta cortisol of <250nmol/L. In contrast, no patients fulfilled the FPC criteria for AI of a peak <33nmol/L (P=0.04 [McNemar test]). In the healthy volunteer cohort, there was one patient each that fulfilled the TPC and FPC criteria for AI.

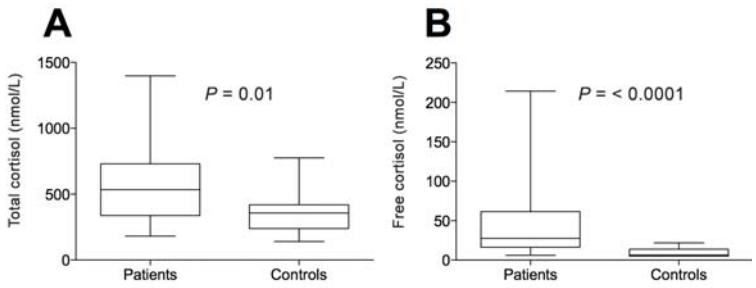
Comparison with calculated values

To investigate the role of a calculated FPC value as a substitute for direct measurement, we compared our observed values with those obtained by calculation from the Coolens equation.[16] Data were available for 36 samples to calculate FPC by the Coolens equation (16 patients and 20 controls). For patients, values estimated using the Coolens equation were significantly different to those determined by direct measurement (57 nmol/L [SD, 36

nmol/L] v 35 nmol/L [SD, 28 nmol/L], respectively [P = 0.0009]), but not for controls (16 nmol/L [SD, 14 nmol/L] v 9.4 nmol/L [SD, 5.8 nmol/L], respectively [P = 0.08]). Measured and calculated FPC values were well correlated and significant for patients (ρ = 81, P = 0.0002) and less well correlated for controls (ρ = 0.44, P = 0.054).

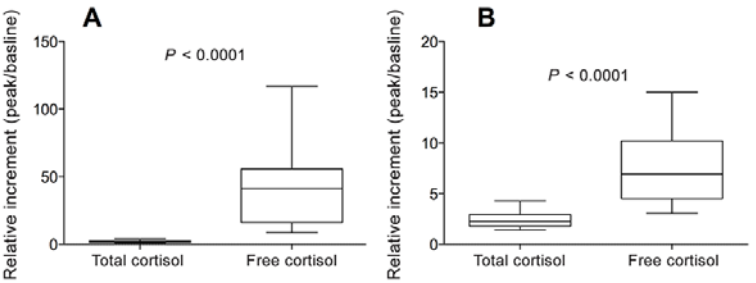
Bias between calculated and measured methods of determining FPC was –22% (SD, 21%) for patients and –6.4% (SD, 15%) for controls. The 95% limits of agreement were from –64% to 20% for patients, and from –36% to 23% for controls.

Figure 1. Box and whisker plots comparing total cortisol and free cortisol at baseline



A: total cortisol concentrations for patients and controls. B: free cortisol concentrations for patients and controls. Boxes are interquartile ranges; horizontal bars are medians; vertical whiskers are 5th to 95th percentiles.

Figure 2. Box and whisker plots comparing the relative increment (calculated as peak/baseline) between total and free cortisol in response to the short Synacthen test



A: patients. B: controls. Boxes are interquartile ranges; horizontal bars are medians; vertical whiskers are 5th to 95th percentiles.

Discussion

The main findings of this study are that TPC and FPC are significantly elevated in patients with aSAH. However, the FPC response was proportionately fivefold greater in magnitude compared with the TPC response. The other key finding was the significant discordance in the incidence of AI using the two sets of diagnostic criteria. When using TPC, the incidence was 30%, in contrast to 0% when using FPC criteria. The FPC increment in response to corticotropin stimulation was significantly larger in patients compared with controls, whereas there was no difference observed in the TPC increment between the groups.

Abnormalities of the HPAA as a chronic consequence of aSAH have been well documented,[17] but there has been relatively less attention paid to the acute phase of injury. Weant and colleagues described an incidence of AI of 69% in patients within 2 weeks of the primary bleed, and noted that baseline TPC concentrations were correlated with the duration of intensive care unit and hospital stay.[5] Similarly, Bendel and colleagues described an incidence of AI in acute aSAH of 17%–50%, but found no correlation between cortisol levels and severity of injury.[3]

Our report highlights the difficulties of diagnosing AI in patients with critical illness. Traditionally, TPC measurements have been used to assess adrenal function. Most of TPC is protein bound, but it is the unbound free portion, representing only about 5%–10% of TPC, that is biologically active. In situations such as critical illness, when protein levels are abnormal, measurements of TPC may be relatively low compared with FPC, leading to an incorrect assumption that adrenal function is impaired.[7] However, techniques to measure FPC are not widely available, so current diagnostic criteria use TPC measurements, despite evidence suggesting that FPC measurements may be more valid.[8]

Using these data, our observations suggest that AI is less prevalent in acute aSAH than previous studies indicate. Our findings show discordance between the response of FPC and TPC to corticotropin stimulation in this group. There is a much greater relative increase in the FPC fraction compared with the TPC, which casts some doubt on the validity of using cortisol increment as a diagnostic criterion for AI; a concern which is reflected in the endocrinological literature.[18] We could find no evidence of AI in our cohort using FPC measurements, in contrast to an observed incidence of 30% when TPC measurements were used.

Earlier studies that have examined FPC levels in aSAH are limited by using calculated values rather than direct measurement. These authors have questioned the use of FPC measurements, given their observations of a high degree of correlation between TPC and calculated FPC values.[3,19]

However, correlation alone is not sufficient to conclude that analytical methods are equivalent; measurement of bias is required.[20] Comparing our measured values with calculated values, we observed a substantial degree of bias, suggesting that these values cannot be used interchangeably, an observation that we have made previously in different populations.[9] Our population of patients with aSAH had significantly lower CBG concentrations than healthy controls, a finding that has been previously observed in brain-injured patients as well as in multitrauma and septic shock, and which may reflect a catabolic process leading to CBG degradation.[21,22]

Strengths and limitations

To our knowledge, this is the first study to directly measure free cortisol concentrations in a population of patients with aSAH, and to compare these values to those from healthy control subjects. Additionally, we were able to measure FPC using ultra-high-performance liquid chromatography tandem mass spectrometry, a validated method which is the gold standard.[11] All patients were steroid-naive, and cortisol measurements were coupled with CBG to provide a clear understanding of FPC profiles and to enable simultaneous calculated values. Data collection was prospective.

There are limitations to the current study that deserve mention. As there were relatively low numbers, the study was not powered for outcome analysis, and may have resulted in a type II error. This study only included one time point, and daily fluctuations and variations over the course of the illness cannot be accounted for. The use of sedative agents may have influenced the results.[4] The control population was younger than the study population. However, while TPC values have been shown to vary with age, the magnitude of these changes is small, so they are unlikely to have contributed to the differences observed between the groups.[23]

We used a standard dose of corticotropin 250 μg, although a low-dose test using corticotropin 1 μg has also been reported. While the low-dose test may be more sensitive, it has not been validated in a critically ill population, for whom the recommendations are that the standard-dose test should be used.[24]

Conclusions

In patients in the acute phase after aSAH, FPC increase is fivefold greater than that of TPC. There is discordance between calculated and measured FPC as well as between the TPC and FPC responses to corticotropin. Using the two fractions to diagnose AI in the same population results in a significant difference in the observed incidence, with no evidence of impaired adrenal function apparent when the FPC fraction is used for measurement. We advocate caution in the assessment of adrenal cortical function using measurements of TPC in this population and the interpretation of the responses to the SST.

Competing interests

None declared.

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