

# Is peri-operative cortisol secretion related to post-operative cognitive dysfunction?

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**Background:** The pattern of cortisol secretion is influenced by surgery. As cortisol can adversely affect neuronal function, this may be an important factor in the development of post-operative cognitive dysfunction (POCD). We hypothesized that the incidence of POCD would be related to changes in cortisol level.

**Methods:** We studied 187 patients aged over 60 years undergoing major non-cardiac surgery with general or regional anaesthesia. Saliva cortisol levels were measured pre-operatively and at 1 day, 7 days and 3 months post-operatively in the morning (08.00 h) and in the afternoon (16.00 h) using salivettes. Cognitive function was assessed pre-operatively, on day 7 and at 3 months using four neuropsychological tests. POCD was defined as a combined Z score of greater than 1.96.

**Results:** After surgery, salivary cortisol concentrations increased significantly. POCD was detected in 18.8% of subjects at 1 week and in 15.2% after 3 months. The pre-operative ratios between the morning and afternoon cortisol concentrations (am/pm ratios) were 2.8 and 2.7 in patients with POCD at 1 week vs. those

without POCD at 1 week, respectively. The am/pm ratios decreased significantly post-operatively to 1.9 and 1.6 at 1 week, respectively ( $P = 0.02$  for both). In an analysis considering all am/pm ratios, it was found that the persistent flattening in am/pm ratio was significantly related to POCD at 1 week.

**Conclusion:** The pattern of diurnal variation in cortisol level was significantly related to POCD. Thus, circadian rhythm disturbance or metabolic endocrine stress could be an important mechanism in the development of cognitive dysfunction after major surgery.

Accepted for publication 10 April 2005

**Key words:** anaesthesia; cognitive function; complications; cortisol; post-operative period; surgery.

© Acta Anaesthesiologica Scandinavica 49 (2005)

MAJOR surgery causes a clear neuroendocrine response with the release of hypothalamic-pituitary-adrenal (HPA) and sympathetic nervous system hormones (1). Cognitive impairment has been associated with high levels of glucocorticoids, as documented in a variety of experimental and clinical settings (2, 3). Post-operative cognitive dysfunction (POCD) is a common complication after

major surgery, especially in elderly patients (4). It has been proposed that stress-inducing stimuli elicit greater activation of the HPA axis in both aged rodents and humans, and a specific finding has been the delayed return to baseline of glucocorticoid levels following a stressor (5). Cortisol has been found to be toxic to cells in the hippocampus, a structure that plays a critical role in the consolidation of short-term into long-term explicit memory as well as descending control of the HPA axis (6). This gave rise to the proposal that repeated episodes of stress cause

\*ISPOCD2 Investigators are listed in the Appendix.

decreased hippocampal inhibition of the HPA axis and thus prolonged hyperactivation (7, 8). As well as a possible causal relationship, changes in cortisol secretion may reflect other metabolic changes. For example, an earlier study by Riis et al. (9) hypothesized that 'increased metabolic demands induced by the endocrine metabolic response to surgery', as measured by cortisol, contribute to POCD.

The aim of this study was to investigate the biological mechanisms underlying post-operative changes in cognition by testing the hypothesis that POCD is related to peri-operative changes in cortisol secretion.

## Materials and methods

Ten hospitals in seven countries contributed patients to the study, each using the same protocol. All patients were aged over 60 years and presented for major surgery with general or regional anaesthesia. In total, 158 patients also participated in another study in which neuropsychological function was assessed, but the data from 29 patients have not been presented previously (10). We enrolled subjects with an expected hospital stay of at least 4 days between October 1998 and October 2000 with follow-up until March 2001. The study received ethics committee approval in all institutions and each participant gave written informed consent.

Exclusion criteria included diseases of the central nervous system (CNS). General anaesthesia was performed according to usual anaesthetist and institution practice, except that normocapnia was maintained in all patients. In the regional anaesthesia group, spinal or epidural anaesthesia was employed and sedation with propofol was permitted.

Salivary cortisol was measured twice a day using salivettes (Sarstedt, Nümbrecht, Germany) given to the patients on four separate days: on the day before surgery, on the first post-operative day, 1 week after surgery (or at discharge, whichever came first) and 3 months later. The saliva samples were obtained at 08.00 h and at 16.00 h ( $\pm 1$  h, i.e. between 07.00 and 09.00 h and between 15.00 and 17.00 h) on each of the 4 days. Patients chewed on a cotton plug for 2–5 min, stimulating saliva production so that the plug absorbed sufficient saliva to become moist. The cotton plugs contained a salivary stimulant and citrate for preservation. The cotton plugs were then placed in plastic containers stored at 4°C or less and shipped by mail to the central laboratory facility for analysis. Here, the salivettes were centrifuged and the samples stored at –20°C until assay.

Salivary cortisol was measured using a  $^{125}\text{I}$  disequilibrium assay for which the radioactive cortisol was supplied by Netria (London, UK) and the primary antibody Ab1002 and the solid phase antirabbit serum by IDS (Bolden, Tyne and Wear, UK). Pools of assays were discarded if the quality controls were greater than two standard deviations from the accepted mean. The intra- and inter-assay coefficients of variation for 7.0, 47 and 87 nmol/l cortisol samples were 7.0% and 11%, 7.0% and 8.4%, and 4.0% and 5.8%, respectively.

For each set of morning and afternoon values, we calculated the ratio between them, the so-called am/pm ratio.

Cognitive function was assessed using neuropsychological testing pre-operatively (baseline) and at 7 days and 3 months post-operatively, comparing the values at baseline with those after surgery (11). The evaluation was based on the following seven variables from the four neuropsychological tests: cumulative number of words recalled in three trials and the number of words at delayed recall from the Visual Verbal Learning Test (12); the time and number of errors in part C of the Concept Shifting Test (13); the time and error scores from the third part of the Stroop Color Word Interference Test (14); and the number of correct answers from the Letter Digit Coding Test (15).

## Statistical analysis

Cortisol saliva concentration is reported as the median with a 25–75% range. Other continuous data are reported with a 5–95% range. Proportions are reported with 95% confidence intervals where appropriate. We compared the cortisol concentrations and am/pm ratios using the Mann–Whitney *U*-test and analysed the changes in cortisol concentrations within the groups by paired *t*-test. Proportions were compared with the chi-squared test.

The neuropsychological test data were analysed as follows: we calculated the changes in performance of the seven test parameters and, using previously collected data from a group of 176 healthy controls (4), we subtracted the average learning effect from these changes and divided the result by the control group standard deviation to obtain a *Z* score for each individual test outcome. Patients had cognitive dysfunction when two out of seven *Z* scores in individual tests or the combined *Z* score was 1.96 or more, a criterion based on our previous study (4).

The association with POCD and the change in cortisol am/pm ratio over time were assessed in a

multivariate linear regression model. In order to assume conditional normality, the cortisol am/pm ratios were log-transformed. For each patient, there were four cortisol am/pm ratios corresponding to the four time points: pre-operative, 1 day after surgery, 1 week after surgery and 3 months after surgery. The pattern was assumed to be different for patients with POCD. The relationship between POCD and cortisol am/pm ratio change was assessed by the *F*-test removing both the POCD main effect and the POCD-time interaction. The association between POCD and the time pattern of cortisol am/pm ratio change was tested by the *F*-test removing only the POCD-time interaction (as the POCD main effect denotes merely a shift in time-average level of the cortisol am/pm ratio). This inherent correlation between the four observations of the cortisol am/pm ratio from the same patient at different time points was modelled as a random effect (SAS PROC MIXED, Cary, NC) (16).

This is represented in the model by a generic level of cortisol am/pm ratio for each patient, assumed to be normally distributed around the estimated mean, i.e. the linear form consisting of the POCD and time indicators.

We estimated that a sample size of 200 would allow the detection of a difference in cortisol concentration corresponding to 0.5 standard deviation with a power of 0.80 at the 0.05 significance level.

## Results

In total, 187 patients were included. Their characteristics are shown in Table 1. Knee replacement was performed in 56, hip replacement in 39, gynaecological procedures in 49, vascular procedures in 21 and other types of surgery (gastrointestinal, urological and others) in the remainder. Two patients received glucocorticoids pre-operatively.

Salivary cortisol concentration was significantly greater at the morning sampling time than at the afternoon sampling time for all time points ( $P < 0.0001$ , Table 2). The salivary concentration increased after surgery and this was statistically significant for both measurements on the first post-operative day and for the afternoon value at 1 week ( $P < 0.0001$ ,  $P < 0.0001$  and  $P = 0.005$ , respectively).

The first post-operative neuropsychological test was performed in 170 patients after a median of 7 days and the second post-operative test in 151 patients after a median of 100 days. Reasons for missing late follow-up were refusal (in 25), testing not possible (in two), death (in one) and other reasons (in eight).

POCD was found in 32 of 170 patients at 1 week (18.8%, [13.2–25.5%]) and in 23 of 151 patients after 3 months (15.2%, [9.9–22.0%]).

In patients over 70 years, POCD was significantly more common at both 1 week and 3 months (27.1% vs. 8.1% and 20.2% vs. 8.1%;  $P = 0.002$  and  $P = 0.04$ , respectively). Duration of anaesthesia over 120 min was also a significant risk factor for POCD at 1 week (18.1% vs. 8.7%,  $P = 0.006$ ). Regional anaesthesia with spinal or epidural block was used in 62 patients. No significant difference could be detected in single cortisol values at any time point between regional and general anaesthesia or between patients with POCD and patients without POCD when a Bonferroni correction was used (Tables 3 and 4).

The cortisol am/pm ratio decreased significantly 1 day after surgery and tended to be lower in patients with POCD at 1 week and 3 months, but no statistically significant difference was found at any time point (Figs 1 and 2). As seen from the figures, the am/pm ratios were not normally distributed.

Table 1

Characteristics of 187 patients undergoing major surgery and neuropsychological testing. Median (5–95% range) except for sex and ASA physical health class.

|                                                                  |                  |
|------------------------------------------------------------------|------------------|
| Age (years)                                                      | 71.8 (61.2–83.6) |
| ASA physical health class                                        |                  |
| ASA I/II/III/IV                                                  | 61/96/27/2       |
| Sex (male/female)                                                | 68/119           |
| Post-operative hospital stay (days)                              | 8 (2–20)         |
| Duration of anaesthesia (min)                                    | 135 (55–227)     |
| Duration of surgery (min)                                        | 100 (35–227)     |
| Interval between operation and first post-operative test (days)  | 7 (4–17)         |
| Interval between operation and second post-operative test (days) | 100 (76–143)     |
| Interval between pre-operative test and operation (days)         | 1 (0–4)          |

Table 2

Cortisol saliva concentration (nmol/l) in 187 elderly patients undergoing major surgery. Median values and 25–75% percentiles.

|                          | Morning                             | Afternoon                          |
|--------------------------|-------------------------------------|------------------------------------|
| Pre-operative            | 14.6 (9.9–22.1)<br><i>n</i> = 187   | 5.6 (2.7–9.6)<br><i>n</i> = 156    |
| First post-operative day | 20.7 (12.1–34.8)*<br><i>n</i> = 134 | 14.3 (5.4–28.0)*<br><i>n</i> = 149 |
| At 1 week                | 13.6 (9.0–23.1)<br><i>n</i> = 142   | 7.0 (4.3–14.2)*<br><i>n</i> = 134  |
| At 3 months              | 12.4 (6.8–18.5)<br><i>n</i> = 124   | 4.3 (2.5–7.4)<br><i>n</i> = 123    |

\*Significant post-operative increase compared with pre-operative value at the same time of day. All morning values are significantly higher than afternoon values.

The logarithm of the am/pm ratio was analysed using a linear interaction model with POCD and time relative to surgery. Inter-patient correlation of the repeated observations was modelled as a random effect for each patient. It was found that the log am/pm ratio changed significantly over time ( $P = 0.004$ ) and that this change was significantly related to POCD at 1 week ( $P = 0.03$  for the POCD–time interaction term). Both the model and inspection of the figures indicate that the development of POCD was significantly related to persistent flattening of the ratio over time. In a model that included POCD at 3 months, only time was significantly related to the change in am/pm ratio ( $P = 0.002$ ), whereas POCD was not ( $P = 0.15$ ).

## Discussion

An expected and significant increase in cortisol level was found after surgery and, also as expected, the

greatest values were found in the morning. The greatest increase was seen in the afternoon levels of cortisol and, consequently, a flattening of the normal diurnal variation was found. This was expressed as a lower post-operative am/pm ratio of salivary cortisol concentration, and the most important finding in this study was the detection of a significant relationship between POCD and the loss of diurnal variation in cortisol level.

The previously documented relationship between the neuroendocrine response to stress and cognitive dysfunction has been developed via a combination of animal studies and some human investigations (2, 3). Standard evaluation of the HPA axis entails the measurement of glucocorticoid levels (cortico-sterone in the rat, cortisol in humans), although adrenocorticotrophic hormone (ACTH) and corticotrophin-releasing factor measurements may be more informative and interpretable. Many of these studies were based on single measurements

Table 3

Cortisol saliva concentration (nmol/l) in elderly patients undergoing major surgery and neuropsychological testing at 1 week ( $n = 170$ ). Median values and 25–75% percentiles.

|                          | Morning                   |                               | Afternoon                 |                               | am/pm ratio<br>POCD1/No POCD1 |
|--------------------------|---------------------------|-------------------------------|---------------------------|-------------------------------|-------------------------------|
|                          | POCD1<br>( <i>n</i> = 32) | No POCD1<br>( <i>n</i> = 138) | POCD1<br>( <i>n</i> = 32) | No POCD1<br>( <i>n</i> = 138) |                               |
| Pre-operative            | 13.8<br>(8.0–23.8)        | 14.7<br>(9.9–22.2)            | 4.8<br>(1.7–10.0)         | 6.2<br>(2.9–9.3)              | 2.8/2.7                       |
| First post-operative day | 28.8<br>(9.2–33.2)        | 20.6<br>(12.2–35.9)           | 12.6<br>(4.8–22.3)        | 15.5<br>(5.6–30.0)            | 1.9/1.6                       |
| At 1 week                | 13.0<br>(8.0–23.4)        | 14.4<br>(9.2–23.0)            | 8.8<br>(6.2–14.9)         | 6.8<br>(4.2–13.5)             | 1.6/2.2                       |
| At 3 months              | 12.5<br>(5.7–14.8)        | 12.6<br>(7.2–19.3)            | 5.2<br>(3.2–8.8)          | 4.2<br>(2.3–7.3)              | 2.0/3.0                       |

POCD1, cognitive dysfunction at 1 week.

Table 4

Cortisol saliva concentration (nmol/l) in elderly patients undergoing major surgery and neuropsychological testing at 3 months ( $n = 151$ ). Median values and 25–75% percentiles.

|                          | Morning               |                           | Afternoon             |                           | am/pm ratio<br>POCD2/No POCD2 |
|--------------------------|-----------------------|---------------------------|-----------------------|---------------------------|-------------------------------|
|                          | POCD2<br>( $n = 23$ ) | No POCD2<br>( $n = 128$ ) | POCD2<br>( $n = 23$ ) | No POCD2<br>( $n = 128$ ) |                               |
| Pre-operative            | 16.5<br>(12.2–25.0)   | 14.0<br>(9.2–21.3)        | 3.8<br>(1.9–12.1)     | 5.4<br>(2.7–9.4)          | 3.3/2.6                       |
| First post-operative day | 18.4<br>(12.1–29.2)   | 22.0<br>(11.8–41.8)       | 11.9<br>(4.2–16.9)    | 14.7<br>(5.2–28.7)        | 1.8/1.8                       |
| At 1 week                | 16.6<br>(9.4–23.0)    | 13.3<br>(8.3–23.1)        | 13.7<br>(6.8–15.5)    | 6.7<br>(4.0–12.9)         | 1.7/2.3                       |
| At 3 months              | 9.9                   | 12.7                      | 6.9                   | 4.2                       | 2.3/2.8                       |

of hormones, thus failing to account for rapid fluctuations in hormone levels. Yet another complicating factor is an alteration of peripheral metabolism of glucocorticoids during ageing.

Human studies provide direct evidence of cognitive impairment following high levels of glucocorticoids. A 4-day treatment with dexamethasone produced a selective impairment of declarative memory, a hippocampal and temporal lobe function (3). Cushing's syndrome is associated with cognitive impairment and hippocampal atrophy that is reversible following the lowering of cortisol levels after treatment (17). Thus, a body of knowledge suggests that the HPA axis response to stress, primarily glucocorticoids, can independently alter cognitive function in a variety of settings.

In the clinical setting, it is much more difficult to assess the possible association between cognitive dysfunction and high levels of glucocorticoids. No sampling method can ever fully capture biologically active fluctuating cortisol levels. We used saliva samples as these have been found to reflect the unbound cortisol concentration in a valid and reliable way (18). In this way, we avoided the need for venepuncture and the sampling resulted in the measurement of non-protein-bound cortisol levels. As an alternative, urinary excretion of cortisol may be used, but this is prone to error in collection and is also dependent on rates of excretion. Salivary sampling is acceptable to patients, economical and it is possible to assess diurnal variability using a few samples a day. Large data sets can be generated

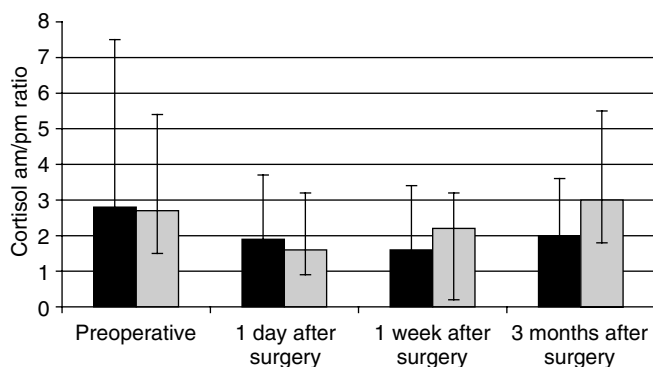


Fig. 1. Cortisol saliva concentration over time shown as the ratio between the value in the morning (am) and in the afternoon (pm). Data on 170 elderly patients who underwent neuropsychological testing before surgery and after 1 week. Dark columns: patients with post-operative cognitive dysfunction at 1 week ( $n = 32$ ). Light columns: patients without cognitive dysfunction at 1 week. The columns represent the median values and the vertical lines indicate the 25–75% range.

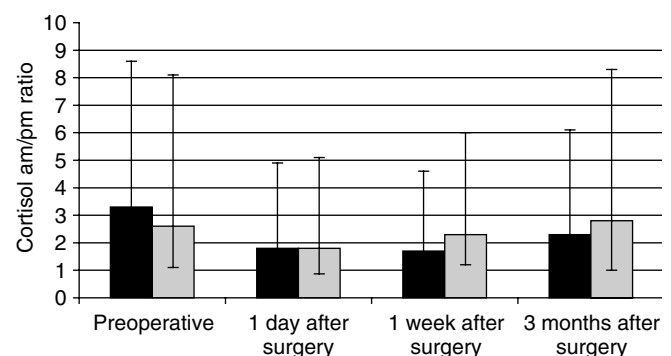


Fig. 2. Cortisol saliva concentration over time shown as the ratio between the value in the morning (am) and in the afternoon (pm). Data on 151 elderly patients who underwent neuropsychological testing before surgery and after 3 months. Dark columns: patients with post-operative cognitive dysfunction at 3 months ( $n = 23$ ). Light columns: patients without cognitive dysfunction at 3 months. The columns represent the median values and the vertical lines indicate the 25–75% range.

and, in multicentre studies involving several countries, such as this one, saliva samples are also easily stored on site and shipped to a central laboratory for greater reliability of analysis. In a study also using salivary cortisol, a flattening of diurnal cortisol was seen in patients with major depression and this was related to a decrement in cognitive speed (19).

The assessment of cognitive function in surgical patients is associated with many important limitations, e.g. the composition of the neuropsychological test battery, the timing of testing and the analysis of test results (11).

Other important limitations were that follow-up neuropsychological testing was not performed in all patients and saliva samples were not always present. This may have led to an inability to detect a significant relationship, because drop out is more likely in patients suffering from post-operative complications.

Several other methods could have been used to assess the association between POCD and the response to major surgery. Hall et al. (20) used blood sampling after hip replacement and noted a significant relationship between functional recovery (walking distance) and markers of inflammation (interleukin-6 and C-reactive protein). Blood concentrations of cortisol and catecholamines were found to correlate less well with outcome, but it should be taken into consideration that all patients in that study received general anaesthesia. Regional anaesthesia inhibits the metabolic stress response, although most effective is continuous epidural infusion of local anaesthetics (21). We found no significant difference in cortisol level between regional and general anaesthesia. This is in accordance with other clinical studies in which central blocks were associated with lower cortisol levels only during and shortly after surgery (22–24). The explanation seems to be that the inhibition of the stress response after regional anaesthesia is rather incomplete and short-lasting after single-dose regional anaesthesia as opposed to continuous epidural infusion.

We found a significant relationship between changes in the am/pm ratio and POCD at 7 days but not at 3 months. Possible explanations include reduced statistical power due to the slightly lower incidence of POCD at 3 months and also the longer period in which other conditions and life events could have influenced cognitive function.

The aetiology of POCD is likely to be multifactorial. In addition to changes in cortisol secretion pattern, other factors, such as an inflammatory response with release of cytokines, may be responsible for changes

in brain function and recovery after surgery. Loss of the diurnal variation of cortisol secretion is associated with sleep deprivation, and altered circadian rhythm of melatonin with disturbed sleep could also be important (25, 26). Patients with POCD may have a more disturbed or irregular pattern of sleep and less ability to cope with daytime stress, thus producing a change in cortisol level. Accordingly, the diminished variation in cortisol could be either a causative or a resultant phenomenon.

In conclusion, the cortisol secretion pattern is profoundly affected (i.e. flattened) by major surgery and this flattening, representing dysfunction of the HPA axis, is significantly related to the occurrence of POCD.

Further studies are needed to assess whether this finding can be confirmed and, if so, whether it is causal, and, more importantly, whether normalization of the HPA axis around the peri-operative period can prevent or ameliorate POCD.

## Acknowledgements

The collaborative parts of the project were funded by a European Union Biomed 2 grant No BMH4-98-3335.

Individual centres received funding from: Denmark: Forskningsstyrelsen, Danish Research Agency, H:S Forskningsfond, Rigshospitalets Jubilæumsfond, Direktør Jacob Madsen og hustrus Olga Madsens Fond, Oberstinde Jensa La Cours Fond, Forskningsfonden for Frederiksborg Amt; Finland: HUS-EVO grants TYH 0051 and TYH 0324; UK: Research into Ageing and BUPA Foundation; Greece: Greek Ministry of Health; Spain: Fondo de Investigaciones Sanitarias (01/0633).

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## Appendix: Participants from the Second International Study of Post-Operative Cognitive Dysfunction (ISPOCD2)

Steering Committee for ISPOCD2: L. S. Rasmussen, C. D. Hanning, H. M. Kuipers, J. Jolles and J. T. Moller.

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