High variability in baseline urinary free cortisol values in patients with Cushing's disease

This is the author's manuscript

Original Citation:

Availability:
This version is available http://hdl.handle.net/2318/143638 since

Published version:
DOI:10.1111/cen.12259

Terms of use:
Open Access
Anyone can freely access the full text of works made available as "Open Access". Works made available under a Creative Commons license can be used according to the terms and conditions of said license. Use of all other works requires consent of the right holder (author or publisher) if not exempted from copyright protection by the applicable law.

(Article begins on next page)
This is the author's final version of the contribution published as:

S. Petersenn; J. Newell-Price; J. W. Findling; F. Gu; M. Maldonado; K. Sen; L. R. Salgado; A. Colao; B. M. K. Biller; Pasireotide B2305 Study Group; E. Ghigo. High variability in baseline urinary free cortisol values in patients with Cushing's disease. CLINICAL ENDOCRINOLOGY. 80 (2) pp: 261-269. DOI: 10.1111/cen.12259

The publisher's version is available at:
http://doi.wiley.com/10.1111/cen.12259

When citing, please refer to the published version.

Link to this full text:
http://hdl.handle.net/2318/143638
High variability in baseline urinary free cortisol values in patients with Cushing’s disease

S. Petersenn*, J. Newell-Price†, J. W. Findling‡, F. Gu§, M. Maldonado¶, K. Sen**, L. R. Salgado††, A. Colao‡‡ and B. M. K. Biller§§ on behalf of the Pasireotide B2305 Study Group

*ENDOC Center for Endocrine Tumors, Hamburg, Germany, †The Medical School, University of Sheffield, Sheffield, UK, ‡Division of Endocrinology, Metabolism, and Clinical Nutrition, Medical College of Wisconsin, Milwaukee, WI, USA, §Department of Endocrinology, Key Laboratory of Endocrinology, Ministry of Health, Peking Union Medical College Hospital, Beijing, China, ¶Clinical Development, Oncology Business Unit, Novartis Pharma AG, Basel, Switzerland, **Oncology Biometrics and Data Management, Novartis Pharmaceuticals Corporation, Florham Park, NJ, USA, ††General Internal Medicine Service, Hospital das Clínicas, University of São Paulo Medical School, São Paulo, Brazil, ‡‡Dipartimento di Medicina Clinica e Chirurgia, Sezione di Endocrinologia, Università di Napoli ‘Federico II’, Naples, Italy and §§Neuroendocrine Clinical Center, Massachusetts General Hospital, Boston, MA, USA

Summary

Objective Twenty-four-hour urinary free cortisol (UFC) sampling is commonly used to evaluate Cushing’s syndrome. Because there are few data on UFC variability in patients with active Cushing’s disease, we analysed baseline UFC in a large patient cohort with moderate-to-severe Cushing’s disease and assessed whether variability correlates with hypercortisolism severity. These data will help clinicians establish the minimum number of UFC samples required to obtain reliable data.

Design Observational study (enrolment phase of Phase III study).

Methods Patients (n = 152) with persistent/recurrent or de novo Cushing’s disease and mean UFC (mUFC) ≥ 1.5 × ULN (normal: 30–145 nmol/24 h) were included. Mean UFC level was calculated from four 24-h urine samples collected over 2 weeks.

Results Over 600 24-h UFC samples were analysed. The mUFC levels of samples 1 and 2 and samples 3 and 4 were 1000 nmol/24 h (SD 1872) and 940 nmol/24 h (SD 2148), respectively; intrapatient coefficient of variation (CV) was 38% for mUFC. The intrapatient CV using all four samples was 52% (95% CI: 48–56). The intrapatient CV was 51% (95% CI: 44–58) for samples 1 and 2, 49% (95% CI: 43–56) for samples 3 and 4 and 54% (95% CI: 49–59) for samples 1, 2 and 3. Variability in mUFC increased as UFC levels increased. There were no correlations between UFC and clinical features of hypercortisolism.

Conclusions There is intrapatient variability of approximately 50% in 24-h UFC measurements, which is relevant to targets set to estimate any treatment effect. Analysing more than two 24-h collection periods in individual patients does not result in a relevant decrease in variability. Interestingly, UFC levels did not correlate with hypercortisolism severity.

(Received 6 March 2013; returned for revision 25 March 2013; finally revised 24 May 2013; accepted 1 June 2013)

Introduction

Cushing’s disease is caused by hypersecretion of adrenocorticotrophic hormone (ACTH) from a corticotroph adenoma,¹,² which in turn causes overproduction of cortisol from the adrenal glands. Patients with Cushing’s disease have a 5-5-fold higher mortality than the general population³ and tend to have numerous comorbidities, including central obesity, osteoporosis, systemic arterial hypertension, insulin resistance, glucose intolerance, diabetes mellitus, dyslipidaemia and cardiovascular disease.⁴,⁵ Early diagnosis and treatment of chronic hypercortisolism are paramount, because a long duration of disease is associated with increasingly severe complications. Effective treatment improves life expectancy in patients with Cushing’s disease.⁶,⁷

The evaluation of a patient with suspected hypercortisolism is often complex and expensive.⁸ The pulsatile nature and circadian variability of ACTH and cortisol secretion in healthy individuals and in patients with Cushing’s syndrome mean that random blood ACTH and cortisol sampling are not useful for diagnosis (or for evaluating a patient during treatment). The Endocrine Society recommends measurement of 24-h urinary free cortisol (UFC), late-night salivary cortisol, or cortisol suppression during a 1-mg overnight dexamethasone suppression test (DST), or
48-h low-dose DST, for the detection of Cushing’s syndrome.\textsuperscript{9} A systematic review and meta-analysis of the literature on diagnostic tests for Cushing’s syndrome showed that UFC and overnight DST have the most evidence to support their use, although the receiver operator characteristic curves for UFC were slightly inferior to those for DST.\textsuperscript{10}

Despite shortcomings, UFC measurements are often used in medical practice to diagnose Cushing’s disease, to assess for remission after pituitary surgery, to evaluate the efficacy of medical treatments and to monitor for recurrence.\textsuperscript{5,11,12} The reliability and reproducibility of this test are very important. The Endocrine Society recommendations state that at least two 24-h UFC measurements should be performed for the diagnosis of Cushing’s syndrome.\textsuperscript{9} It is, however, currently unclear whether two 24-h collections are sufficient to establish a reasonable estimate of the secretory activity of the underlying pituitary adenoma or whether more than two measurements are required. In addition, the level of intra-individual variability when using this test is not known.

The aim of this study was to use the four baseline UFC measurements taken from each patient in the randomized, double-blind, Phase III study of pasireotide (NCT00434148)\textsuperscript{13} to quantify the intrapatient variability of UFC levels in patients with Cushing’s disease. In addition, the study aimed to show whether a greater number of UFC measurements leads to greater accuracy of UFC measurement and more precise estimation of intrapatient variation and whether UFC levels correlate with the severity of clinical features of hypercortisolism.

**Methods**

**Patients and study design**

Men and women aged ≥18 years with persistent/recurrent or de novo (if not surgical candidates) Cushing’s disease were eligible for enrolment into this international, multicentre study.\textsuperscript{15} Patients had a confirmed diagnosis of Cushing’s disease defined as: a mean UFC (mUFC) level at least 1.5 times the upper limit of the normal range (ULN) calculated from four 24-h urine samples collected within 2 weeks; morning plasma ACTH within the normal range or above the ULN; and either magnetic resonance imaging confirmation of a pituitary macroadenoma (≥10 mm), inferior petrosal sinus sampling (IPSS) gradient >3 after corticotropin-releasing hormone (CRH) stimulation for patients with a microadenoma (<10 mm) or histopathology confirming an ACTH-staining adenoma.\textsuperscript{14} If IPSS had previously been performed without CRH (e.g. with desmopressin),\textsuperscript{15} then a central to peripheral prestimulation gradient >2 was required. If IPSS had not previously been performed, IPSS with CRH stimulation was required. Only patients with four baseline UFC samples were included in the present analysis.

Patients were excluded from the clinical trial if they had received pituitary irradiation within the last 10 years (patients were eligible if they had been treated more than 10 years before enrolment), had been treated with mitotane during the 6 months prior to visit 1 or had optic chiasm compression causing any visual field defect or Cushing’s syndrome due to nonpituitary sources or an inherited syndrome. In patients who had received previous medical treatment for Cushing’s disease, the following washout periods were employed before baseline UFC was assessed: 1 week for steroidogenesis inhibitors (ketonconazole, metyrapone), octreotide sc and rosiglitazone; 4 weeks for dopamine agonists (bromocriptine, cabergoline) and lanreotide SR; and 8 weeks for octreotide LAR and lanreotide Autogel.

This Phase III study was approved by the independent ethics committee, research ethics board or institutional review board at each centre and complied with the ICH Harmonised Tripartite Guidelines for Good Clinical Practice, the Declaration of Helsinki and local laws. All patients provided written informed consent.

**UFC measurements and other assays**

At baseline (visit 1), four 24-h urine samples were collected over a 2-week period and were used to calculate the mUFC level for each patient; each collection took place from 0800 h to 0800 h the following day. Patients were given both written and verbal instructions for collecting and storing the urine samples. The first morning urine was not collected on day 1, but was included on the second day, ending the 24-h collection period. Urinary analyses took place at central laboratories, and the volume for each collection was noted. UFC was measured in duplicate by high-performance liquid chromatography (HPLC) as described previously,\textsuperscript{16} using the Alliance\textsuperscript{8} 2795 High Throughput System (Waters Corp, Milford, MA, USA). The normal range for this assay is 30–145 nmol/24 h (approximately 11–53 μg/24 h), which was determined based on an analysis of 24-h urine samples from 28 healthy individuals.\textsuperscript{16} The intra-assay variability was 6–1% at a concentration of 5 nmol/l, 5–5% at 15 nmol/l, 1–5% at 75 nmol/l, 3–7% at 400 nmol/l and 0–9% at 2000 nmol/l. The interassay precision was 5–7% at 15 nmol/l, 2–4% at 75 nmol/l, 2–4% at 400 nmol/l and 4–6% at 2000 nmol/l. The interassay precision was not calculated at 5 nmol/l. The total accuracy was 100 ± 15%. All samples were analysed by central laboratories [Eurofins Medinet BV, Breda, The Netherlands, for Europe; CRL Medinet Inc, Lenexa, KS, USA, for North/South America; and Eurofins Technology Services (Suzhou) Co Ltd, Suzhou, China, for China]. All three laboratories were consistent in the procedures used.

Height, weight, blood pressure, fasting glucose, insulin, glycosylated haemoglobin (HbA1C) and creatinine levels were also collected. Body mass index (BMI), insulin resistance [homeostasis model assessment (HOMA) IR] and β-cell function (HOMA β) were calculated.

**Statistical methods**

The degree of UFC variability was determined from the four urine samples collected from individual patients. The naïve estimator of the true intrapatient coefficient of variation (CV) obtained as the average of the intrapatient CV for individual patients has been found to underestimate the true intrapatient
Therefore, a logarithmic transformation was applied to the UFC values before estimating the intrapatient variability using a variance components model. Intrapatient CV was calculated as follows: intrapatient CV = antilog [intrapatient standard deviation (SD) on the log scale] −1. The effect of baseline UFC levels (i.e. ≤2 vs >2×ULN and ≤5 vs >5×ULN) and disease status (i.e. de novo vs recurrent/persistent) on intrapatient UFC variability was evaluated by estimating the CV (as described above) for these subgroups. Analyses regarding the effect of multiple UFC samples (four vs three vs two) on intrapatient UFC variability were also performed.

The impact of averaging multiple UFC samples on the precision of the UFC assessment was evaluated by deriving the intrapatient SD obtained by the average of multiple UFC samples. For the two-sample case, the intrapatient CV was also empirically determined by averaging samples 1 and 2 separately from samples 3 and 4 for all patients with four samples. The 97.5% lower confidence bound of the relative difference between two baseline UFC assessments of the same patient was computed to determine a threshold beyond which an intervention effect could be considered highly unlikely to be due to random UFC variation. The computations were performed for the average of two, three and four samples, assuming log-normal distribution of the UFC values.

A linear correlation analysis between baseline UFC and baseline fasting glucose, HbA1c, fasting insulin, fasting HOMA IR, HOMA β, BMI and sitting systolic and diastolic blood pressure was performed to assess the relationship between UFC level and the severity of clinical features of Cushing’s disease. Pearson correlation coefficients are reported.

**Results**

**Baseline demographics and characteristics**

Of the 162 patients who entered the study, 152 (93-8%) had four UFC measurements at baseline. The baseline demographic and disease characteristics of these 152 patients are presented in Table 1.

Most patients had persistent/recurrent Cushing’s disease and had undergone surgical intervention but not pituitary irradiation. Almost all patients (98%) reported at least one continuing comorbidity which was hypertension (78-0% of patients), osteoporosis (22-8%), diabetes mellitus (33-9%), hypothyroidism (20-4%), hypercholesterolaemia (18-0%), hyperlipidaemia (16-7%) and depression (16-0%).

As shown in Table 2, mean baseline UFC was 972 ± 1985 nmol/24 h (352 ± 720 μg/24 h). Most patients had moderate-to-severe hypercortisolism (>2×ULN; Fig. 1), and almost 50% of patients had a mUFC level ≥4×ULN. It should be noted that prior to a protocol amendment, UFC was assessed at screening (two samples) and again at baseline (study day 1; four samples); one patient included in this analysis was enrolled before the amendment and had an eligible mUFC level at screening but an mUFC level that was <1.5×ULN when assessed at baseline.

### Table 1. Demographic and disease characteristics

| Characteristics | All patients with four baseline UFC measurements (N = 152) |
|-----------------|-----------------------------------------------------------|
| Age, years | Mean ± SD 40.3 ± 11.9 |
| Sex, n (%) | ≥65, n (%) 5 (3.3) |
| Race, n (%) | Caucasian 119 (78-3) |
| Time since diagnosis, months | Mean ± SD 53.2 ± 63.0 |
| Cushing’s disease status, n (%) | Median (range) 29-7 (0-1-372.1) |
| Persistent/recurrent | 25 (16-4) |
| Previous surgery, n (%) | 127 (83-6) |
| Previous pituitary irradiation, n (%) | 121 (79-6) |
| Previous medication for Cushing’s disease, n (%) | 7 (4-6)* |
| Mean baseline urinary creatinine, nmol/24 h | 972 ± 1985 |
| Baseline mUFC (mean of four UFC samples) | Mean ± SD, μg/24 h 352 ± 720 |
| Median (range), nmol/24 h | 561 (195–22 944) |
| Median (range), μg/24 h | 203 (71–8316) |
| Mean baseline urinary volume, ml/24 h | 9.5 ± 3-1 |
| Hypercortisolism severity, n (%) | Mean ± SD, mg/24 h 1076 ± 354 |
| Mild (mUFC ≤2×ULN) | 26 (17-1) |
| Moderate (mUFC >2–5×ULN) | 66 (43-4) |
| Severe (mUFC >5–10×ULN) | 40 (26-3) |
| Very severe (mUFC >10×ULN) | 20 (13-2) |

SD, standard deviation; *Two of the seven patients received pituitary irradiation within 10 years prior to study entry.

### Table 2. Baseline UFC, creatinine levels and urinary volumes

| Characteristics | Patients (N = 152) |
|-----------------|------------------|
| Baseline mUFC (mean of four UFC samples) | Mean ± SD, nmol/24 h 972 ± 1985 |
| Mean ± SD, μg/24 h | 352 ± 720 |
| Median (range), nmol/24 h | 561 (195–22 944) |
| Median (range), μg/24 h | 203 (71–8316) |
| Mean baseline urinary volume, ml/24 h | 2121 ± 909 |
| Hypercortisolism severity, n (%) | Mean ± SD, mg/24 h 1076 ± 354 |
| Mild (mUFC ≤2×ULN) | 26 (17-1) |
| Moderate (mUFC >2–5×ULN) | 66 (43-4) |
| Severe (mUFC >5–10×ULN) | 40 (26-3) |
| Very severe (mUFC >10×ULN) | 20 (13-2) |

mUFC, mean urinary free cortisol in an individual patient; SD, standard deviation; ULN, upper limit of normal (145 nmol/24 h; 53 μg/24 h).
Baseline UFC variability

The individual baseline UFC levels exhibited non-normal distribution, due to the mean cortisol threshold of $\geq 1.5 \times$ ULN required for inclusion into the study. Seven patients had one or more individual UFC values in the normal range, despite the mean of their four UFC values being $\geq 1.5 \times$ ULN. Four of these patients had one value in the normal range, and three patients had two normal values. As shown in Fig. 2, for the group as a whole, the mean and median UFC levels from UFC samples 1 and 2 were similar to those collected from samples 3 and 4. However, the intrapatient CV between the mean of samples 1 and 2 and the mean of samples 3 and 4 was 38%.

When individual patient data were examined, the variability in UFC levels increased as UFC levels decreased (Fig. 3a). This was supported by the analysis of variability according to baseline UFC category (calculated using all four samples), where patients with UFC $\leq 2 \times$ ULN had an intrapatient CV of 35% [95% confidence interval (CI): 29–42; $n = 26$] compared with 55% [95% CI: 50–60; $n = 126$] in those with UFC $> 2 \times$ ULN. Similarly, patients with UFC $\leq 5 \times$ ULN had an intrapatient CV of 45% [95% CI: 41–50; $n = 92$] compared with 62% [95% CI: 54–71; $n = 60$] in those with UFC $> 5 \times$ ULN. There was a correlation between the first two baseline UFC samples from the same patient (Fig. 3b). No apparent trend over time was evident in the variability from the first to the last sample (Fig. 3c). The widest intrapatient range was 600–14 020 nmol/24 h (217.5–5081.5 µg/24 h). In the 25 patients with de novo Cushing’s disease, the intrapatient CV (calculated using all four samples) was 38% [95% CI: 31–46] compared with 55% [95% CI: 50–60] in the 127 patients with persistent/recurrent disease.

The overall intrapatient CV calculated using all four baseline UFC samples was 52% [95% CI: 48–56]. The intrapatient CV calculated using two or three UFC measures were similar but had slightly wider CIs. The intrapatient CV was 51% [95% CI: 44–58] when samples 1 and 2 were used, 49% [95% CI: 43–56] when samples 3 and 4 were used and 54% [95% CI: 49–59] when samples 1, 2 and 3 were used.

The intrapatient CV of baseline UFC assessments is high; therefore, it is desirable to average at least two UFC samples to accurately estimate a patient’s baseline UFC level. While averaging two UFC samples is expected to reduce the CV to 34.6% (consistent with the 38% reported earlier as the intrapatient CV for the mean of samples 1 and 2 and the mean of samples 3 and 4), averaging three and four UFC samples is expected to reduce the CV further to 27.5% and 23.4%, respectively. An intrapatient CV of 34.6% implies that there is a 97.5% chance that one baseline UFC measurement could be lower than another baseline measurement of the same patient by at most 56%. In other words, the reduction in UFC from baseline would have to be greater than 56% to be considered highly unlikely to be due to random variation in UFC levels. Similarly, an intrapatient CV of 27.5% (when averaging three UFC samples) and 23.4% (when averaging four UFC samples) implies that the reduction in UFC from baseline would have to be greater than 49% and 44%, respectively, to be considered highly unlikely to be due to random variation in UFC levels.

Correlation of baseline mUFC with clinical features of Cushing’s disease

There was no linear correlation between baseline mUFC and fasting glucose, HbA1C, fasting insulin, insulin resistance, β-cell function, BMI or systolic/diastolic blood pressure (Fig. 4).
Fig. 3 (a) Variability of urinary free cortisol (UFC) levels in relation to the mean UFC for individual patients (mUFC); (b) Correlation between UFC samples 1 and 2 from individual patients; (c) UFC levels from the first, second, third and fourth collections. Note: The inserts in (a) and (b) show the same figure but with the extreme outliers removed and the axis scales expanded. Twelve patients had at least one UFC value >2500 nmol/24 h. Each data point in (a), (b) and each line in (c) represents an individual patient (N = 152).
Fig. 4 Correlation between the mean UFC for individual patients (mUFC) and clinical features of Cushing’s disease: (a) fasting glucose; (b) HbA1c; (c) fasting insulin; (d) insulin resistance (HOMA IR); (e) β-cell function (HOMA β); (f) body mass index (BMI); (g) sitting systolic blood pressure; and (h) sitting diastolic blood pressure. Note: The shaded areas represent the normal ranges for UFC (x-axis) and each individual clinical feature (y-axis).
Discussion

This study shows that in patients with moderate-to-severe hypercortisolism, the intrapatient CV across two or more UFC samples is approximately 50% and that variability increases with progressively higher UFC levels. This is important because it is clinically valuable for physicians to be aware that there can be a high degree of variability in UFC levels in any individual patient. Interestingly, patients with de novo Cushing’s disease had lower variability in baseline UFC than those with persistent/recurrent disease; the reason for this difference is unclear. Patients with persistent/recurrent disease may have a more aggressive form of tumour with a different pattern of ACTH secretion compared with de novo patients. In addition, it is possible that previous surgery has altered the structure of the underlying pituitary tumour, and any remnant/recurrence of this leads to a different ACTH secretion pattern. Several extrinsic factors have been shown to modify the excretion of UFC, including alcoholism, polycystic ovarian syndrome and anorexia nervosa. Patients with a recent history of alcohol abuse were excluded from the study and there were no patients with a history of anorexia, although eight female patients with active polycystic ovarian syndrome were enrolled. The intrapatient CV between the mean of UFC samples 1 and 2 and the mean of UFC samples 3 and 4 was lower, but was still 38%. Overall, UFC was lower in samples 3 and 4 than in samples 1 and 2, but this is unlikely to be a washout phenomenon because the UFC samples could have been taken at any point within the 2-week period and there was no clear trend for UFC levels to decrease throughout the 2-week period. Although the reproducibility of late-night salivary cortisol has been measured previously, to our knowledge, this is the first such analysis of the intrapatient variance of 24-h UFC measurements. This study is unique because it was conducted in a large patient population that provided over 600 UFC samples for evaluation. These analyses demonstrate the wide variations in UFC measurements that occur over a short period of time within individual patients and have important implications for the diagnosis of Cushing’s disease and the assessment of treatment efficacy. These data imply that for a patient who has moderate-to-severe Cushing’s disease, reductions in UFC from baseline (based on the average of two UFC samples) should be greater than 56% for an intervention effect to be considered beyond the normal variability of UFC.

The data can also be used to evaluate how many UFC measurements should be collected when evaluating Cushing’s disease. In this study, a two-sample 24-h UFC measurement was found to yield a reasonable estimate of intrapatient variation (95% CI: 44–58%). Narrower CIs, and therefore more precise estimates, were obtained when using three (95% CI: 49–59%) or four (95% CI: 48–56%) UFC measurements. However, practical considerations in clinical practice may prevent the routine collection of four 24-h UFC samples, because patients may find the task inconvenient. The results of this study therefore suggest that in this range of cortisol excess, taking the mean of two samples would provide sufficient information for use in everyday care.

No linear correlation was found in the present study between mUFC levels and the severity of features of hypercortisolism, including fasting glucose, HbA1C, fasting insulin, insulin resistance, β-cell function, BMI or systolic/diastolic blood pressure. This is perhaps surprising because it might be expected that higher UFC levels would correlate with disease severity and therefore with morbidity levels. Previous studies have shown that higher UFC levels in patients with Cushing’s disease are associated with more severe cognitive impairment, the presence of major depression and the risk of serious infection. It is, however, possible that disease severity is associated with duration rather than degree of hypercortisolism, and it may also be the case that a linear correlation could not be demonstrated because patients with milder degrees of cortisol excess were not included in this study. The terms of the study protocol did not allow for anything more than the basic screening log details (e.g. patient initials, date examined, reason for exclusion) to be collected for patients who were excluded; as such, we are unable to evaluate the effect of including patients with milder cortisol excess. In addition, the lack of correlation may be linked to differences between patients in their sensitivity to excess cortisol. Further research is required to clarify these findings and explore the existence of nonlinear relationships.

It is important to consider the patient population enrolled into this study and the relatively short time period of this analysis (2 weeks) when interpreting the results. The data presented here were generated from an analysis of the four baseline 24-h UFC measurements that were taken from each patient during the screening period of the largest, prospective, interventional trial of a medical therapy in patients with Cushing’s disease. Eligibility in this 12-month, Phase III trial of pasireotide was assessed using the mean of three or four UFC samples taken within 2 weeks; even greater variability could be expected over longer time intervals. Although a UFC level ≥1.5 × ULN was required for inclusion into the study, the overall mean UFC level at entry was approximately 6.7 × ULN, and just 7 of 152 patients had individual UFC values in the normal range. Additional studies are required to show whether the observed variability would also be applicable in patients with lower or much higher UFC levels. This is particularly important because this study found that intrapatient variability in UFC levels increased as UFC levels increased. It is therefore possible that patients with mild hypercortisolism have less variability in UFC levels than these patients with moderate-to-severe hypercortisolism. It is also possible that some of the observed variability is a reflection of the inherent difficulties with urine collection and UFC measurement. Although UFC was analysed at three different central laboratories, they all used the same assays and adhered to stringent quality control procedures. Furthermore, samples from single patients were all measured in the same laboratory, so the variability results should not be affected. However, no cross-validation between the laboratories was performed, which is a potential limitation of the study. Similarly, we do not believe that the observed variability was influenced by previous treatment regimens. Although allowed by the inclusion criteria, none of the enrolled patients received prior medical treatment with long-acting somatostatin analogues. Nineteen patients had previously been treated with cabergoline at some time point; however, with a half-life of around 3 days and a protocol-specified washout period of at least 4 weeks, this should...
not have affected the results. These factors further indicate the need to study additional parameters, such as late-night salivary cortisol, to assess their value in the diagnosis and monitoring of patients with Cushing’s disease.

It would also be interesting to assess the correlation between UFC values and urinary creatinine levels in patients with Cushing’s disease. It might be expected that 24-h urine creatinine levels below the lower limit of normal would reflect insufficient collection, which would in turn affect the accuracy of the UFC results. However, some patients with Cushing’s disease have relatively low creatinine levels because of muscle wasting.\(^{27}\) Additional studies are therefore required to assess whether there is indeed a negative correlation between urinary creatinine and UFC levels. In addition, because some drugs (fenofibrate and carbamazepine) may artificially increase urine cortisol when measured by HPLC, urine cortisol levels should be interpreted cautiously in patients receiving these agents.\(^{28,29}\)

In conclusion, 24-h UFC measurements in this large cohort of patients with moderate-to-severe hypercortisolism show high intrapatient variability. The clinically important results demonstrate that in these patients, a two-sample UFC measurement yields a reasonable estimate of intrapatient variation, but that more precise estimates can be obtained with three or four UFC measures. Based on these results, we suggest that a minimum of two samples be obtained for diagnosis, in agreement with the Endocrine Society’s guidelines,\(^{9}\) as well as for assessing efficacy of treatment.

Acknowledgements
We thank Ulrike Schoenherr for her contributions to the conduct of the CSOM230B2305 study. We also thank all investigators who contributed to the study as well as the numerous nurses, study coordinators and patients. Members of the Pasireotide B2305 Study Group included the following: R. Abs, B. Allolio, R. Auchus, J. Bertherat, B. M. K. Biller, C. Boguszewski, M. Boscaro, T. Brue, O. Bruno, P. Caron, D. Carvalho, F. Cavagnini, O. Chabre, A. Chervin, C. Chik, A. Colao, A. Côrmealçi, C. Cortet, M. Czepielewski, E. Delegi Uberti, A. Estour, U. Feldt-Rasmussen, M. Fleseriu, P. Freda, M. Gadelha, M. Gershinsky, E. Ghigo, B. Glaser, F. Gu, M. Guitelmann, S. Guler, A. Hamrahian, S. A. Imran, Z. Jin, J. O. Jorgensen, P. Kadioglu, L. Katzenelson, A. Lacroix, A. Leal Cerro, P. Loli, L. Lu, W. Ludlam, F. Mantero, J. Martins, M. Mercado, A. Moreira, G. Ning, K. Nogueira, L. Østergaard Kristensen, S. Petersenn, G. Piaditis, L. Portocarrero, V. Rohmer, R. Rubens, L. Salgado, S. Samson, J. Schopohl, I. Shimon, C. Strasburger, A. Tabarin, M. Terzolo, S. Tsegarakis, N. Ungar, Z. Valkusz, M. Wälimäki, L. Van Gaal, E. Vidal, S. Webb, R. Weiss and W. Zgliczyński.

Disclosures
S. Petersenn has worked on an advisory board for Novartis and has received lecture fees from Novartis, Ipsen and Pfizer; J. Newell-Price has received research grants, consultancy fees and speaker fees from Novartis; J. W. Findling has worked as a consultant on advisory committees for Novartis; F. Gu has received research grants from Novartis; L. R. Salgado has received honoraria from Novartis; A. Colao has received research grants and lecture fees and has worked on advisory boards for Novartis; in the past 2 years, B. M. K. Biller has served as the Principal Investigator on studies with research grants from Novartis and Corcept to the Massachusetts General Hospital Neuroendocrine Unit and as a consultant to Novartis and HRA Pharma. K. Sen is an employee of Novartis. M. Maldonado was an employee of Novartis during this study and is now employed by Boehringer Ingelheim.

Funding
This study was funded by Novartis Pharma AG. Financial support for medical editorial assistance was provided by Novartis Pharmaceuticals Corporation. We thank Keri Wellington, PhD, for medical editorial assistance with this manuscript.

References
1 Bertagna, X., Guignat, L., Grousset, L. et al. (2009) Cushing’s disease. Best Practice and Research Clinical Endocrinology and Metabolism, 23, 607–623.
2 Newell-Price, J., Bertagna, X., Grossman, A.B. et al. (2006) Cushing’s syndrome. The Lancet, 367, 1605–1617.
3 Clayton, R.N., Raskauskienė, D., Reulen, R.C. et al. (2011) Mortality and morbidity in Cushing’s disease over 50 years in Stoke-on-Trent, UK: audit and meta-analysis of literature. Journal of Clinical Endocrinology and Metabolism, 96, 632–642.
4 Webb, S.M., Badia, X., Barahona, M.J. et al. (2008) Evaluation of health-related quality of life in patients with Cushing’s syndrome with a new questionnaire. European Journal of Endocrinology, 158, 623–630.
5 Etxabe, J. & Vazquez, J.A. (1994) Mortality and morbidity in Cushing’s disease: an epidemiological approach. Clinical Endocrinology, 40, 479–484.
6 Dekkers, O.M., Biermasz, N.R., Pereira, A.M. et al. (2007) Mortality in patients treated for Cushing’s disease is increased, compared with patients treated for nonfunctioning pituitary macroadenoma. Journal of Clinical Endocrinology and Metabolism, 92, 976–981.
7 Swearingen, B., Biller, B.M., Barker, F.G. et al. (1999) Long-term mortality after transsphenoidal surgery for Cushing disease. Annals of Internal Medicine, 130, 821–824.
8 Boscaro, M. & Arnaldi, G. (2009) Approach to the patient with possible Cushing’s syndrome. Journal of Clinical Endocrinology and Metabolism, 94, 3121–3131.
9 Nieman, L.K., Biller, B.M., Findling, J.W. et al. (2008) The diagnosis of Cushing’s syndrome: an Endocrine Society clinical practice guideline. Journal of Clinical Endocrinology and Metabolism, 93, 1526–1540.
10 Elamin, M.B., Murad, M.H., Mullan, R. et al. (2008) Accuracy of diagnostic tests for Cushing’s syndrome: a systematic review and metaanalyses. Journal of Clinical Endocrinology and Metabolism, 93, 1553–1562.
11 Pivonello, R., De Martino, M.C., Cappabianca, P. et al. (2009) The medical treatment of Cushing’s disease: effectiveness of chronic treatment with the dopamine agonist cabergoline in
patients unsuccessfully treated by surgery. Journal of Clinical Endocrinology and Metabolism, 94, 223–230.
12 Godbout, A., Manavela, M.P., Danilowicz, K. et al. (2010) Cabergoline monotherapy in the long-term treatment of Cushing’s disease. European Journal of Endocrinology, 163, 709–716.
13 Colao, A., Petersenn, S., Newell-Price, J. et al. (2012) A 12-month Phase 3 study of pasireotide in Cushing’s disease. The New England Journal of Medicine, 366, 914–924.
14 Oldfield, E.H., Doppman, J.L., Nieman, L.K. et al. (1991) Petrosal sinus sampling with and without corticotropin-releasing hormone for the differential diagnosis of Cushing’s syndrome. The New England Journal of Medicine, 325, 897–905.
15 Malerbi, D.A., Mendonca, B.B., Liberman, B. et al. (1993) The desmopressin stimulation test in the differential diagnosis of Cushing’s syndrome. Clinical Endocrinology, 38, 463–472.
16 Turpeinen, U., Markkanen, H., Valimaki, M. et al. (1997) Determination of urinary free cortisol by HPLC. Clinical Chemistry, 43, 1386–1391.
17 Quan, H. & Shih, W.J. (1996) Assessing reproducibility by the within-subject coefficient of variation with random effects models. Biometrics, 52, 1195–1203.
18 Besemer, F., Pereira, A.M. & Smit, J.W. (2011) Alcohol-induced Cushing syndrome. Hypercortisolism caused by alcohol abuse. The Netherlands Journal of Medicine, 69, 318–323.
19 Invitti, C., Pecori Giraldi, F., Dubini, A. et al. (1991) Increased urinary free cortisol and decreased serum corticosteroid-binding globulin in polycystic ovary syndrome. Acta Endocrinologica (Copenhagen), 125, 28–32.
20 Vierhapper, H., Kiss, A., Nowotny, P. et al. (1990) Metabolism of cortisol in anorexia nervosa. Acta Endocrinologica (Copenhagen), 122, 753–758.
21 Viardot, A., Huber, P., Puder, J.J. et al. (2005) Reproducibility of nighttime salivary cortisol and its use in the diagnosis of hypercortisolism compared with urinary free cortisol and overnight dexamethasone suppression test. Journal of Clinical Endocrinology and Metabolism, 90, 5730–5736.
22 Starkman, M.N., Giordani, B., Berent, S. et al. (2001) Elevated cortisol levels in Cushing’s disease are associated with cognitive decrements. Psychosomatic Medicine, 63, 985–993.
23 Sonino, N., Fava, G.A., Raffi, A.R. et al. (1998) Clinical correlates of major depression in Cushing’s disease. Psychopathology, 31, 302–306.
24 Bakker, R.C., Gallas, P.R., Romijn, J.A. et al. (1998) Cushing’s syndrome complicated by multiple opportunistic infections. Journal of Endocrinological Investigation, 21, 329–333.
25 Graham, B.S. & Tucker, W.S. Jr (1984) Opportunistic infections in endogenous Cushing’s syndrome. Annals of Internal Medicine, 101, 334–338.
26 Del Dotto, P. & Bonuccelli, U. (2003) Clinical pharmacokinetics of cabergoline. Clinical Pharmacokinetics, 42, 633–645.
27 Pecori Giraldi, F., Moro, M. & Cavagnini, F. (2003) Gender-related differences in the presentation and course of Cushing’s disease. Journal of Clinical Endocrinology and Metabolism, 88, 1554–1558.
28 Findling, J.W., Pinkstaff, S.M., Shaker, J.L. et al. (1998) Pseudo-hypercortisoluria: spurious elevation of urinary cortisol due to carbamazepine. The Endocrinologist, 8, 51–54.
29 Meikle, A.W., Findling, J., Kushnir, M.M. et al. (2003) Pseudo-Cushing syndrome caused by fenofibrate interference with urinary cortisol assayed by high-performance liquid chromatography. Journal of Clinical Endocrinology and Metabolism, 88, 3521–3524.

© 2013 The Authors. Clinical Endocrinology published by John Wiley & Sons Ltd. Clinical Endocrinology (2014), 80, 261–269