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# Evaluation of Late-Night Salivary Cortisol during a Phase III Study with Pasireotide in Patients with Cushing's Disease

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## INTRODUCTION

- The measurement of 24-hour urinary free cortisol (UFC) levels is used for the diagnosis[1] and subsequent monitoring of patients with Cushing's disease. Limitations of measuring UFC include inadequate urine collection, high intra-patient variability and a lack of immunoassay specificity.[2–4]
- Alternative measurement methods include serum or salivary cortisol levels.
- High concordance has been shown between late-night salivary cortisol (LNSC) and UFC when screening for Cushing's syndrome;[5] less is known about its use for monitoring treatment.
- The measurement of salivary cortisol is a simple and convenient technique with high sensitivity and specificity.[6,7] Cortisol levels in saliva are independent of salivary flow rates and are stable at room temperature.[8,9]
- However, samples taken in the outpatient setting may be affected by extrinsic factors such as food, exercise or various emotional/physical disturbances and there is currently a lack of standardized immunoassays.[10,11]
- The current analysis in patients with Cushing's disease evaluates LNSC levels and their association with UFC levels and clinical signs and symptoms during treatment with the multireceptor-targeted somatostatin analogue pasireotide (Signifor®).

## METHODS

### Patients and Dosing

- Patients enrolled in this 12-month study were aged ≥18 years with confirmed *de novo* (if not surgical candidates) or persistent/recurrent Cushing's disease, defined by a mean UFC level ≥1.5 times the upper limit of normal (ULN).
- Patients were randomized to receive pasireotide 600 µg or 900 µg sc bid. Dose increases (up to 1200 µg bid) were allowed after month 3; reductions in steps of 300 µg bid for drug-related adverse events (AEs) were permitted throughout the study.

### Assessments

- The primary endpoint was the proportion of patients with UFC <ULN at month 6 without prior dose increase. UFC levels were assessed monthly for 6 months and every 3 months thereafter.
- UFC values were determined by high-performance liquid chromatography (Alliance® 2795 High Throughput System, Waters Corp, Milford, MA, USA; normal range 30–145 nmol/24h; limit of quantification 5 nmol/L; intra-assay variability of 0.9–6.1% at 5–2000 nmol/L; inter-assay precision of 2.4–5.7% at 15–2000 nmol/L) and all samples were analyzed by central laboratories
  - UFC control was defined as levels ≤ULN, partial control as levels >ULN and ≥50% reduction from baseline, and uncontrolled UFC as levels >ULN and <50% reduction from baseline.
- LNSC evaluation was an exploratory endpoint based on a single, optional measurement at midnight (±1 hour) taken on the same day as one of the 24-hour UFC measurements. LNSC was measured using enzyme-linked immunosorbent assay (RE52611, IBL-Hamburg GmbH, Germany; normal range 0.83–8.3 nmol/L; limit of detection 0.41 nmol/L; intra-assay variability of 3.2–7.6% at 7.0–80.8 nmol/L; inter-assay variability of 6.2–9.1% at 5.9–72.8 nmol/L) at baseline and after 3, 6 and 12 months of treatment.
- Sitting systolic (SBP) and diastolic blood pressure (DBP), weight, body mass index (BMI) and total cholesterol were assessed monthly; information regarding dose adjustments of antihypertensive or lipid-lowering medication was not collected. Waist circumference, health-related quality of life (HRQoL; assessed using CushingQoL) and depression status (based on Beck Depression Inventory II [BDI-II]) were measured at months 3, 6 and 12.

### Statistical Analysis

- Only patients with available LNSC measurements at baseline were included (n=93).
- Correlation between LNSC and UFC was evaluated in an exploratory manner using Spearman's rank correlation. At each time point (ie baseline, months 3, 6, 9 and 12), only patients with both UFC and LNSC assessments within the same 24-hour period were included.
- Changes in clinical signs and symptoms were evaluated according to LNSC response at month 6. Responders were patients with LNSC levels ≤ULN; if LNSC levels at month 6 were missing, the value was imputed using the last available measurement between months 3 and 6 inclusive.

## RESULTS

### Baseline Characteristics

- Baseline LNSC levels were available in 93 patients; 48 were randomized to pasireotide 600 µg bid and 45 were randomized to pasireotide 900 µg bid
  - Median baseline LNSC levels were 17.3 and 10.3 nmol/L, respectively.
- Of these 93 patients, 26 (28.0%) had normal LNSC levels at baseline (median level of 5.7 nmol/L).
- Sixty-seven patients (72.0%) had baseline LNSC levels >ULN (n=40 in the pasireotide 600 µg bid group and n=27 in the pasireotide 900 µg bid group; **Table 1**)
  - Median LNSC levels were 19.7 and 20.7 nmol/L in the 600 µg and 900 µg dose groups, respectively.

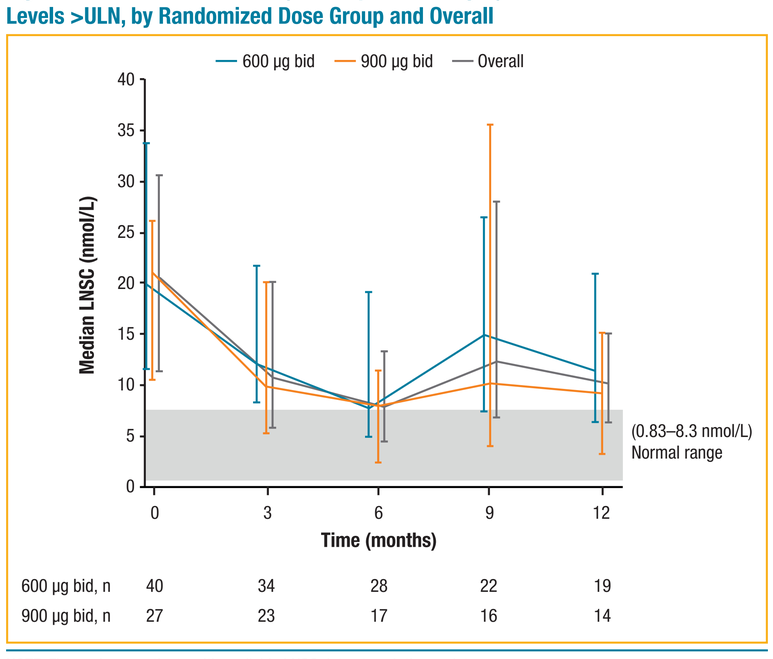
**Table 1. Patient Demographics and Characteristics at Baseline in 67 Patients with Baseline LNSC >ULN**

| Demographic variable | Pasireotide 600 µg bid (n=40) | Pasireotide 900 µg bid (n=27) |
|---|---|---|
| Median age, years | 37.5 | 41.0 |
| Male:female | 9:31 | 4:23 |
| Race, n (%) | | |
| Caucasian | 27 (67.5) | 18 (66.7) |
| Black | 0 | 1 (3.7) |
| Asian | 9 (22.5) | 7 (25.9) |
| Other | 4 (10.0) | 1 (3.7) |
| Median time since diagnosis (months) | 23.6 | 30.8 |
| Previous surgery, n (%) | 29 (72.5) | 21 (77.8) |
| Median LNSC level, nmol/L | 19.7 | 20.7 |

### Effect of Pasireotide Treatment on LNSC Levels

- In the group of patients with available LNSC measurements at baseline, LNSC levels decreased by a median of 4.9 nmol/L (–26.5%; n=34) and 2.4 nmol/L (–41.8%; n=28) in the 600 µg and 900 µg groups, respectively, after 6 months of pasireotide treatment. Overall median LNSC decreases after 12 months of treatment were 7.2 nmol/L (–42.2%; n=24) and 1.6 nmol/L (–26.1%; n=21), respectively.
- In the patients who had baseline LNSC levels >ULN, median LNSC levels decreased from baseline to month 12 during pasireotide treatment (**Figure 1**). Median percentage LNSC changes in the 600 µg and 900 µg groups were –34.2% and –63.8% after 6 months of pasireotide treatment and –52.6% and –56.1% after 12 months of treatment (**Table 2**).
- The equivalent median changes in patients with baseline LNSC ≤ULN were +23.4% (n=6) and +8.1% (n=11) at month 6, and +70.4% (n=5) and +37.8% (n=7) at month 12.

**Figure 1. Median LNSC Levels (± Interquartile Ranges) in 67 Patients with Baseline Levels >ULN, by Randomized Dose Group and Overall**




NOTE: Figure shows patients with available LNSC data at each time point

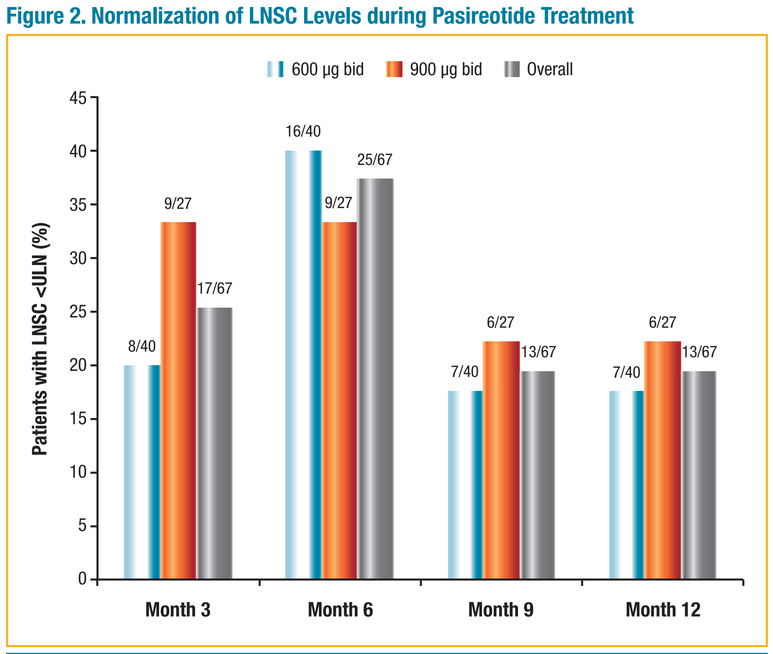
**Table 2. Change from Baseline in LNSC after 6 and 12 Months of Pasireotide Treatment in 67 Patients with Baseline LNSC Levels >ULN**

| | Pasireotide 600 µg bid (n=40) | Pasireotide 900 µg bid (n=27) | Overall (n=67) |
|---|---|---|---|
| Baseline LNSC, nmol/L Median (range) | 19.7 (9–553) | 20.7 (9–550) | 20.4 (9–553) |
| Change at month 6, nmol/L Median (%; range %) | (n=28) –6.8 (–34.2; –89, 545) | (n=17) –12.1 (–63.8; –100, 110) | (n=45) –8.1 (–53.6; –100, 545) |
| Change at month 12, nmol/L Median (%; range %) | (n=19) –13.4 (–52.6; –89, 115) | (n=14) –11.8 (–56.1; –97, 73) | (n=33) –11.9 (–52.6; –97, 115) |

### Normalization of LNSC in Patients with Baseline Levels >ULN

- LNSC levels had normalized by month 6 in 25/67 patients (37.3%; **Figure 2**)
  - Ten of the 25 patients with normalized LNSC also had UFC control, while seven patients had partial UFC control.
- At month 12, LNSC levels had normalized in 13/67 patients (19.4%; **Figure 2**)
  - Of these 13 patients, eight and four also had UFC control and partial control, respectively.
- In both dose groups, median LNSC levels had decreased at 12 months in patients with controlled (–46.8% in 600 µg group, n=5; –29.4% in 900 µg group, n=14) and partially controlled (–71.6% in 600 µg group, n=9; –81.4% in 900 µg group, n=2) UFC at month 6, and increased in uncontrolled patients (48.9% in 600 µg group, n=10; 33.1% in 900 µg group, n=5).

**Figure 2. Normalization of LNSC Levels during Pasireotide Treatment**

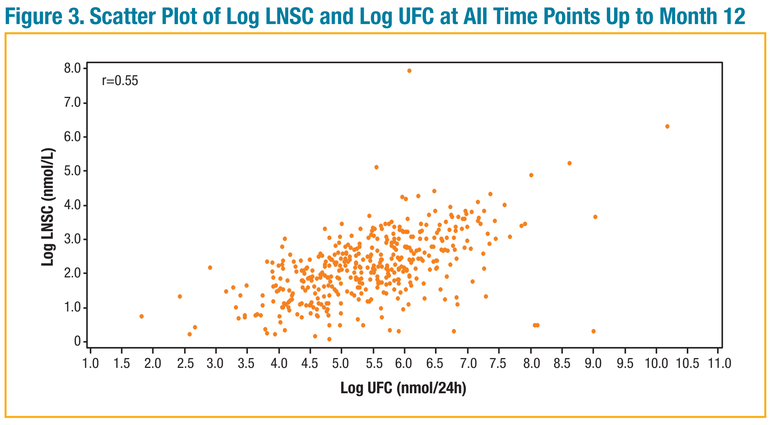



NOTE: Patients with missing data at any time point are counted as non-normalizers (except at month 6 where there is some imputation as stated in the statistical analysis section)

### Correlation Between LNSC and UFC

- The Spearman's rank correlation between LNSC and UFC was r=0.45 at baseline. Following 6 and 12 months of pasireotide treatment, the correlation was r=0.58 and r=0.33, respectively.
- When all time points were pooled, the Spearman's rank correlation was r=0.55 (**Figure 3**).

**Figure 3. Scatter Plot of Log LNSC and Log UFC at All Time Points Up to Month 12**




### Effect of Pasireotide Treatment on Clinical Signs and Symptoms

- Reductions in SBP, DBP, BMI and weight were generally observed at months 6 and 12, regardless of LNSC responder status (**Table 3**).
- Improvements in waist circumference, HRQoL and BDI-II were observed in both LNSC response subgroups at months 6 and 12 (except for an increase in waist circumference in LNSC responders at month 6)
  - Greater improvements were observed in LNSC non-responders for waist circumference and HRQoL.
- Reductions in total cholesterol levels at months 6 and 12 were similar in the two responder subgroups.

**Table 3. Changes from Baseline at Months 6 and 12 in Clinical Signs and Symptoms, by LNSC Response Status**

| | Mean change at month 6 ± SD | | Mean change at month 12 ± SD | |
|---|---|---|---|---|
| | LNSC responder | LNSC non-responder | LNSC responder | LNSC non-responder |
| Sitting SBP, mmHg | –8.8 ± 19.2 (n=24) | –10.7 ± 17.6 (n=25) | –9.8 ± 21.0 (n=20) | 0.0 ± 13.7 (n=16) |
| Sitting DBP, mmHg | –3.2 ± 16.4 (n=24) | –7.1 ± 12.6 (n=25) | –4.2 ± 14.4 (n=20) | –0.9 ± 8.0 (n=16) |
| BMI, kg/m$^2$ | –1.3 ± 1.6 (n=24) | –1.8 ± 1.6 (n=25) | –2.2 ± 2.2 (n=20) | –1.6 ± 2.0 (n=16) |
| Weight, kg | –3.4 ± 4.2 (n=24) | –4.7 ± 4.2 (n=25) | –5.9 ± 5.8 (n=20) | –4.4 ± 5.5 (n=16) |
| Waist circumference, cm | 1.3 ± 7.5 (n=21) | –6.9 ± 7.3 (n=24) | –2.9 ± 10.1 (n=18) | –7.0 ± 8.7 (n=15) |
| Total cholesterol, mmol/L | –0.2 ± 1.3 (n=24) | –0.3 ± 1.1 (n=25) | –0.4 ± 1.5 (n=20) | –0.3 ± 1.3 (n=16) |
| HRQoL | 4.5 ± 14.3 (n=23) | 8.2 ± 17.7 (n=24) | 3.5 ± 14.4 (n=19) | 10.6 ± 20.9 (n=15) |
| BDI-II | –5.6 ± 9.3 (n=23) | –3.8 ± 9.4 (n=24) | –4.6 ± 10.0 (n=19) | –1.1 ± 9.2 (n=16) |

## CONCLUSIONS

- **An overall decrease in LNSC was observed during 12 months of pasireotide treatment, similar to the overall decrease in UFC levels.[12] Decreases were generally greater in patients with baseline LNSC >ULN.**
- **Following 6 months of pasireotide treatment, LNSC levels normalized in 37% of patients with baseline levels >ULN (n=25/67); most also had achieved UFC control or partial control.**
- **The Spearman's rank correlation between LNSC and UFC was relatively consistent at baseline and throughout the pasireotide treatment period. However, it should be noted that these correlations were based on single salivary cortisol samples.**
- **Improvements in clinical signs and symptoms of Cushing's disease were noted even without complete LNSC normalization.**
- **Salivary cortisol is a simpler and more convenient biomarker than 24-hour UFC. It may have value in diagnosing and assessing treatment response in patients with Cushing's disease.**

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