



SALC-A 2023 included a case describing a 54-year-old woman with signs and symptoms of hypercortisolism and laboratory testing suggestive of Cushing's Syndrome (CS). Both the 24-hour urinary free cortisol and cortisol after administration of 1 mg of dexamethasone were elevated. However, a late-night (sometimes referred to as "midnight") salivary cortisol, performed one week after this initial testing, was normal. We asked how you would investigate the discrepancy between the salivary cortisol result and the other test results, and gave you the following choices:

- Repeating the late-night salivary cortisol test
- Performing a high-dose dexamethasone suppression test
- Measuring plasma ACTH
- Performing a corticotropin releasing hormone test

Nineteen participants responded to the Dry Challenge and the overwhelming majority (74%) chose repeating the late-night salivary cortisol test. The remainder were divided between a high-dose dexamethasone suppression test (16%) and a plasma ACTH (10%). None chose corticotropin releasing hormone testing.

Guidelines for the diagnosis of CS call for performing one or more of the following tests: 24-hour urinary free cortisol, low-dose dexamethasone suppression, or late-night salivary cortisol ¹. Because this patient's signs and symptoms are so consistent with hypercortisolism and the results of the other testing overwhelmingly support the diagnosis of CS, chosen by most of the participants, the late-night salivary cortisol result should be suspected of being a false-negative and repeated.

Cortisol is normally secreted in a pulsatile manner. It rises during the early hours of the day post ACTH-stimulation and falls in the evening. Because the late-night salivary cortisol sample is collected just before an overnight rest (at which point cortisol should be at its lowest point or nadir), the test is very sensitive to the loss of this circadian rhythm. Late-night salivary cortisol results may be misleading if patients do not collect the sample properly. But this was not the case for our patient, and a key clue is the fact that the salivary cortisol test was done one week after the other testing.

Although the normal circadian rhythm of cortisol secretion is usually lost in CS, some variability in the pattern of secretion may still be present. One large study over the course of several weeks has shown that salivary cortisol testing in CS patients can reveal peaks and valleys of secretion ². There is even a rare syndrome called "cyclical" CS, with periods of milder symptoms associated with normal cortisol levels ³. In our patient, a repeat salivary cortisol test produced a positive result (5 ng/mL, with a cut-off of <2).

When faced with a normal test result that seems inconsistent with the rest of the other test results and clinical presentation, repeating the test is usually a good idea and the first line of investigation.



References

1. Nieman K, Biller BM, Findling JW, et al.: The diagnosis of Cushing's syndrome: An Endocrine Society clinical practice guideline. *J Clin Endocrinol Metab* 2008; 93:1526-1540
2. Sandouk Z, Johnston P, Bunch D, et al: Variability of late-night salivary cortisol in Cushing disease: A prospective study. *J Clin Endocrinol Metab* 2018; 103:983-990
3. An M, Hendricks L, Bachrach. Diagnostic challenges and considerations of cyclical Cushing's syndrome in a 15-year-old female. *Pediatr Endocrinol Diabetes Metab* 2020; 26:104-107

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