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Pitfalls in the diagnosis and differential diagnosis of Cushing's syndrome*

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In this issue, Thynne and colleagues courageously present the misadventures of a Cushingoid patient's evaluation. The initial evaluation proceeded according to previous guidelines for making the diagnosis of Cushing's syndrome: exogenous steroid use was sought (and prednisone use was affirmed 2 years earlier), and two screening tests for Cushing's syndrome were obtained.¹ These showed only a mild elevation in late-night salivary cortisol (13 and 15 nM, RR < 13 nM), but a very high urine free cortisol (UFC) on two occasions (3281 nmol/24 h (RR 50-350 nmol/24 h). Although not recommended as a screening test, a morning cortisol was within the normal range (410-480 nM (RR 200-700 nM). We do not know the interval between these samples or whether they were obtained simultaneously, but they are wildly discrepant. If they were obtained simultaneously, one or more of them were incorrect: it is not possible to have a urine cortisol that is nearly 10-fold increased with a normal serum cortisol and only mildly increased late-night salivary cortisol. If the samples were collected at intervals and not together, the results suggest cyclic Cushing's syndrome or a strange UFC result. Because tests for the differential diagnosis of Cushing's syndrome may be interpreted to suggest Cushing's disease in normal individuals, those with mild or cyclic ectopic or adrenal causes, it is imperative to first establish the diagnosis unequivocally. Thus, it is likely that further screening tests would be helpful.

The patient was evidently diagnosed as having endogenous Cushing's syndrome, as she underwent differential diagnostic tests: adrenal and chest CT scans and inferior petrosal sinus sampling. Again, we do not know the order of tests, but one consensus group suggested that patients should undergo adrenal imaging if the ACTH level is low and IPSS if the levels are inappropriately normal or high.² Because of radiation exposure, IPSS results should dictate the need for a chest CT to identify a neuroendocrine tumour. In this case, ACTH levels were at the lower end of normal: 13 ng/l (RR 10-60 ng/l, Immulite 2000 XP, Siemens, Sydney, Australia.). However, the functional limit of detection in this assay is too high to discriminate true suppression/undetectable ACTH levels (other assay lower RR are 5 ng/l), and so, values in this range should be considered indeterminate.

During the IPSS, peripheral ACTH levels were <10–16 ng/l, again in a low range. However, the left petrosal sinus showed a response as high as 438 ng/l after CRH, which yielded a central-to-peripheral ACTH ratio of about 29. Such a ratio is consistent with Cushing's

*Please see related paper on pages 328–332 of this issue.

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disease, except when cyclic or mild hypercortisolism is insufficient to suppress normal corticotrophs. In the latter situation, IPSS results of normal individuals, cyclic CS patients and CD patients overlap.^{3,4}

Because early neurosurgical intervention was not available, the patient commenced ketoconazole treatment. Surprisingly, the morning cortisol became subnormal (88 mM) but the UFC remained elevated (3682 mmol/24 h). This discrepancy led to an evaluation for abnormal protein binding or a hook effect in the cortisol assay, both of which were unrevealing. However, high-pressure liquid chromatography–tandem mass spectrometry (HPLC-MS/MS) revealed a subnormal serum and urine cortisol, but high prednisolone concentrations.

This very interesting case illustrates a number of important clinical points. First, it is important to understand the vagaries of assays that we use. Here, the ACTH level was low for Cushing's disease. Measurable but low ACTH in ACTH-independent conditions has been described with other radioimmunoassays and immunoassays, not only by the Pecori Geraldini reference cited,⁵ but also by Invitti *et al.*⁶, who reported on assays with a similarly high detection limit. This suggests that only assays with a lower functional limit of detection should be used. Cross-reactivity of other glucocorticoids (in this case prednisolone) in UFC immunoassays can lead to a marked discrepancy between immunoassay and HPLC-MS/MS results. The authors raise the possibility of CBG abnormalities – this is an important confounder when serum cortisol levels are high and UFC and salivary free measurements are low or normal.

Second, we can avoid some mistakes if we compare multiple simultaneous measures of hypercortisolism. In this case, measurement of serum, salivary and urine cortisol on the same day might have immediately suggested the need for a different UFC assay. Coupled with the low normal ACTH, exogenous glucocorticoid exposure would be the most likely cause. In general, all measurements should be similarly high, normal or low. One exception is the use of MS/MS for the measurement of UFC and immunoassay for measurement of salivary cortisol. In patients with mild hypercortisolism, UFC may be normal when salivary cortisol is elevated, presumably because of a loss of cross-reactivity of metabolites.⁷

Finally, the scheduling and interpretation of the IPSS must include an assessment of the duration and amount of hypercortisolism. IPSS is useful only if normal corticotrophs are suppressed.⁸ The lack of peripheral rise in ACTH after CRH is also a clue that this patient did not have Cushing's disease. A central-to-peripheral ratio of >2 or >3 before or after CRH does not unequivocally establish a diagnosis of CD, especially if salivary and plasma cortisol concentrations are not elevated.

I commend the authors for sharing their experience so that we can all learn from this case.

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