The short Synacthen test and laboratory assay interference

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Case

Michael, 50 years of age, presented with increasing dizziness that was aggravated by standing, although there was no postural hypotension. His general practitioner (GP) requested testing for plasma thyroid-stimulating hormone (TSH) levels to exclude hypothyroidism, and plasma adrenocorticotrophic hormone (ACTH) and cortisol to exclude primary adrenal insufficiency. His medical history included hypertension and type 2 diabetes mellitus. Michael's test results are shown in Table 1.

TSH and cortisol were measured on a Beckman Coulter UniCel DxI analyser. ACTH was measured on a Siemens Immulite analyser. Given the high plasma ACTH and borderline low cortisol levels, Michael's GP ordered a short Synacthen test (SST), which showed normal serum cortisol levels post-Synacthen:

- baseline cortisol (8.10 am): 278 nmol/L
- post-Synacthen cortisol (9.15 am): 624 nmol/L (normal >550 nmol/L)].

Normal SST virtually excludes adrenal insufficiency. The plasma ACTH that was repeated as part of the SST remained elevated at 40 pmol/L. The discordance between plasma ACTH and SST results raised the suspicion of ACTH assay interference.

Question 1

What is the role of basal plasma ACTH and cortisol in the investigation of primary adrenal insufficiency?

Question 2

What is involved in an SST?

Question 3

What are the possible mechanisms for an ACTH assay interference?

Answer 1

Basal plasma cortisol is a viable first step in the investigation of suspected adrenal insufficiency. If the plasma cortisol is >370 nmol/L (dependent on local laboratory cortisol assay parameters), adrenal insufficiency is unlikely.[1] Apart from this, if there is ongoing clinical suspicion, an SST is generally required to exclude adrenal insufficiency.[2] It is important not to miss adrenal insufficiency and hypothyroidism, as significant morbidity and mortality have been reported as a result of missed diagnoses.[3,4]

Answer 2

SST is also known as the cosyntropin test or ACTH test. Synacthen is a trade name for tetracosactrin, a synthetic peptide that comprises the first 24 (out of 39) amino acids of the endogenous ACTH peptide. In an SST, blood for baseline plasma cortisol and ACTH is first collected. Then, 250 µg (adult dose) of Synacthen is administered intramuscularly, followed by blood sampling at 30 minutes and/or 60 minutes for measurement of cortisol levels post-Synacthen.[5] As there are significant variations among different cortisol assays, the cut-off for a normal cortisol response must be laboratory-specific or assay-specific (ranging from 420 to 574 nmol/L).[6]

Synthetic glucocorticoids such as prednisolone and methylprednisolone can potentially cross-react with the routine cortisol immunoassays; therefore, these medications should be stopped 24–48 hours prior to the SST for meaningful interpretation of results.[7] The oral contraceptive pill (OCP) invariably increases plasma total cortisol, probably by increasing cortisol binding globulin. If the OCP cannot be safely withheld, a different post-Synacthen cortisol cut-off should be used (eg 430 nmol/L for women not taking an OCP and 577 nmol/L in those taking it, when measured on an Abbott Architect analyser).[6]

Table 1. Michael's test results

Tests	Results	Reference interval
TSH	2.30 mIU/L	0.30–5.00 mIU/L
Cortisol (8:35 am)	230 nmol/L	240–620 nmol/L
ACTH	40 pmol/L	0–10 pmol/L

ACTH, adrenocorticotrophie hormone; TSH, thyroid stimulating hormone

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Answer 3

Assay interferences have complicated patient care and led to unnecessary investigations and interventions.[8,9] Many hormones, including ACTH, are measured by immunoassays. A typical 'sandwich' chemiluminescent immunoassay has two antibodies to capture the antigen (analyte) of interest. Anything that affects the bridging of the two antibodies can cause falsely higher or lower results. For example, heterophile antibodies are endogenous, weakly reactive antibodies that are reported to be prevalent in up to 40% of the general population.[9] A macro-analyte is an analyte bound to an immunoglobulin (eg macroprolactin or macro-TSH). It remains in the human circulation longer because of reduced clearance. Macro-analytes are biologically inactive but falsely elevate the result.[10] There are several simple procedures the local laboratory can perform:

- Check the result on an alternative analyser in another laboratory.
- Use serial dilutions of the serum/plasma sample.

If the result on an alternative analyser is similar to the initial result, and a serial dilution study shows linearity in the analyte concentrations with increasing dilution, assay interference is unlikely. On the other hand, a different result on an alternative analyser and non-linearity on serial dilution should lead to several more investigations, such as a heterophilic-blocking tube (HBT) study (to exclude heterophile antibodies interference) and a polyethylene glycol precipitation study (to exclude macro-analyte interference).[10]

Conclusion

In Michael's case, a 10-times dilution of his plasma produced a recovery of 825% in ACTH measurements (neat: 40 pmol/L; 10x dilution: 330 pmol/L), which demonstrated non-linearity. The plasma ACTH result on an alternative platform (Roche Cobas analyser) was 8 pmol/L (reference interval: 1.6–13.9 pmol/L). After incubation of the patient's plasma with HBT, the ACTH measurement changed significantly (ACTH post-HBT: 52 pmol/L), which suggests heterophile antibodies interfered with the local laboratory's ACTH assay.

For ongoing patient care, it should be documented in the patient's medical record that the ACTH result from the local laboratory is likely to be unreliable for this patient, and an alternative laboratory for this test should be considered. While the low ACTH level on the alternative platform suggested assay interference on the Beckman Coulter UniCel DxI analyser, other possibilities must be considered in cases of unexpected ACTH levels. Given the notable instability of ACTH, specimen degradation might have contributed to lower ACTH result in the second laboratory if:

- the EDTA whole blood or plasma specimen was not maintained at 4°C (or frozen)
- there was a delay between analysis at the primary laboratory and the secondary laboratory
- an incorrect specimen type (eg clotted serum) was analysed.

In addition, while it is not relevant in our patient's case, there is known inter-assay variability in ACTH measurement, which may contribute to a lower result on one platform versus another.

Michael's dizziness resolved a few weeks later without an obvious cause found.

Key points

- If there is clinical suspicion of adrenal insufficiency, a short Synacthen test is a definitive test to diagnose or exclude the disease.
- If patients are on steroids that can potentially cross-react on cortisol assays, these should be withheld for 24–48 hours prior to the short Synacthen test. Central (hypothalamic/pituitary) suppression due to exogenous steroids is another reason for withholding steroids prior to testing for adrenal insufficiency.
- The cut-offs for the serum cortisol post-Synacthen should be assay-specific.
- The astute clinician is the key to identifying possible assay interferences. Close communication between the clinician and laboratory is essential.

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