

patients a pressure ulcer was identified against the PEG balloon. The histopathological study discarded malignancy, and no *Helicobacter* type bacteria were observed. Although the cases were few, the complication proved serious.

Pressure ulcers associated with tubes of this kind had already been previously described: initially in isolated cases, though they were followed by larger series in 2002 and 2012, which reflected a greater risk of gastric ulcer. Following the demonstration of a 2.27-fold increase in the risk of pressure ulcers, an evaluation of the patient series was made and the administrative procedures for the required changes were implemented.

It can be concluded that UDB secondary to a pressure ulcer caused by a gastrostomy tube with the distal tip outside the balloon is a rare but serious complication. Ensuring patient safety is a key consideration when choosing among the different types of enteral nutrition gastrostomy tubes.

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Analytical interference in the corticotropin immunoassay in patients with adrenal adenomas



Interferencia analítica en el inmunoensayo de corticotropina en pacientes con adenomas suprarrenales

Analytical interference in the corticotropin (ACTH) assay is an uncommon event (<1%).¹ However, when it occurs, it can interfere with the diagnostic orientation and management of adrenal disease leading to inappropriate clinical decisions, especially when patients show alterations in imaging tests.

Case #1

A 79-year-old woman was referred for a right adrenal mass (23 × 16 mm) incidentally discovered in a thoracic-abdominal CT scan performed after trauma. The patient had always had cats. She did not report tachycardia, palpitations, headaches or hyperhidrosis, and did not show

any clinical sign of hypercortisolism. Hyperpigmentation was absent.

Hormone analysis showed marked hypercorticotropinemia (ACTH 242 pg/ml; normal range, *N*: 5–46) with serum cortisol (16.1 mcg/dl; *N*: 3.7–19.4), nighttime (23:00) salivary cortisol (0.1 mcg/dl; *N* < 0.28), and 24-h urinary free cortisol (UFC, 40 mcg/24 h; *N*: < 140) within the normal range. Mineralocorticoid function [aldosterone 4.4 ng/dl (*N*: 3–35.5), plasma renin activity, PRA 1.78 ng/ml/h (*N*: 0.3–7.0)] and medullary adrenal function [24-h urinary metanephrines 168 mcg/24 h (*N*: 50–825)] were also normal. A second plasma ACTH determination confirmed hypercorticotropinemia (ACTH 311 pg/ml).

The presence of hypercorticotropinemia with normal values of serum, urinary and night salivary cortisol in the absence of clinical adrenal dysfunction forced us to rule out ACTH dependent Cushing syndrome (ACTH-dependent CS) and Addison's disease. We performed a 1-mg dexametasone (23:00 h) suppression test (serum cortisol 1.4 mcg/dl; *N* < 1.8) and a short ACTH (250 mcg iv) stimulation test (serum cortisol at 0, 30, and 60 min: 12.8, 21.3, and 23.3 mcg/dl). Antiadrenal antibodies were also negative. A normal pituitary MRI and a negative ^{99m}Tc-EDDA/HYNIC-TOC scintigraphy ruled out the presence of a silent

Table 1 Plasma ACTH (pg/ml) concentrations in plasma samples of the patients after using different analyzers.

	Immulite 2000, Siemens	Immulite 2000, Siemens + HBT, scantibodies, Shantee, CA	Liaison, Diasorin
<i>Patient # 1</i>			
ACTH (pg/ml) sample # 1	552	–	4.4
ACTH (pg/ml) sample # 2	302	142	5.2
<i>Patient # 2</i>			
ACTH (pg/ml)	187	109	6.5

pituitary corticotropinoma and a neuroendocrine tumor, respectively.

The diagnosis of a nonfunctioning adrenal incidentaloma with hypercorticotropinemia not associated with endogenous hypercortisolism or adrenal insufficiency was established. In suspicion of possible antibody interference we pretreated plasma sample of the patient with a heterophilic antibody blocking tube (HBT, Scantibodies, Shantee, CA) that captures potential heterophilic antibodies before hormone quantification. Basal ACTH after HBT remained still high (142 pg/ml). Then ACTH was determined in the same plasma sample with other chemiluminiscent immunometric assay (Liaison, Diasorin) different from the first analyzer used (Immulite 2000, Siemens Healthcare Diagnostics), resulting in the low-normal range (4.4 pg/ml) with the first analyzer while it was disproportionately high with the latter (552 pg/ml). These results were confirmed using another separate plasma sample (5.2 pg/ml and 302 pg/ml with Liaison and Immulite 2000, respectively) (Table 1). According with these results, low-normal plasma ACTH concentrations might be associated with a slight autonomous hypersecretion of cortisol by the adenoma.

These findings were compatible with an analytical interference in ACTH measurement associated with Immulite 2000 analyzer. With the aim of determining other possible antibody interference we performed a serum protein electrophoresis for quantifying the three major classes of immunoglobulins (IgG, IgA, and IgM), which were normal. Lastly, the quantification of rheumatoid factor (RF) in the patient's serum was clearly high (RF 129 IU/ml; $N < 40$), suggesting a possible antibody interference with this immunoglobulin.

Case #2

A 71-year-old woman was referred for follow-up of a papillary thyroid carcinoma. She referred no contact with animals. An abdominal CT scan performed for follow-up of her neoplasia detected bilateral adrenal nodules (15 mm right and 11 mm left).

Initial hormone investigations showed normal urine metanephrines (120 mcg/24 h), serum aldosterone (13 ng/dl), and PRA (2.46 ng/ml/h). Her baseline cortisol (15.7 mcg/dl) and dehydroepiandrosterone sulfate (199 ng/ml) levels were also normal. However, her plasma ACTH level was strikingly high (251 pg/ml). A second plasma ACTH determination in the Immulite instrument (Siemens) confirmed hypercorticotropinemia (ACTH 187 pg/ml). To

complete the hormonal study we carry out the following investigations: urinary free cortisol, 32.5 mcg/24 h; salivary cortisol at 23:00, 0.13 mcg/dl; and serum cortisol after 1 mg dexamethasone, 2.10 mcg/dl. Plasma ACTH concentration measured on Immulite was 109 pg/ml after HBT. In this sample plasma ACTH quantified in the Liaison instrument (Diasorin) was 6.49 pg/ml. The patient had normal serum immunoglobulin concentrations, and the RF test was negative (< 20 U/ml). Results of this patient suggest a slight hypersecretion of cortisol (subclinical hypercortisolism) with low serum ACTH and overnight 1 mg dexamethasone suppression test slightly above the normal value (1.8 mcg/dl).

ACTH measurement after serial dilutions of plasma samples at 1/5 and 1/10 in both patients revealed a concentration that exceeded the coefficient of variation (10%) of this method, suggesting a possible interference. Moreover, the post-polyethylene glycol recovery resulted in undetectable plasma ACTH levels not only in both patients but also in a control plasma sample.

ACTH analytical interference has been occasionally reported mainly associated to Siemens Immulite analyzers.¹⁻⁴ Interfering antibodies can be heterophilic antibodies, human anti-animal antibodies (HAAA), and RF.^{5,6} Analytical interference in the first patient could be attributable to the presence of RF in plasma. However, in the second one the cause remains undetermined.

RF is present in about 5–10% of the general population and in up to 70% of patients with rheumatoid arthritis, although it may precede the disease for many years. In addition, it can appear in a large number of autoimmune systemic diseases, in various infectious diseases, and in many inflammatory conditions and malignancies.⁷ RF can produce falsely elevated levels when immunometric methods such as enzyme-linked immunosorbent assay (ELISA) or multiplex immunoassays (MIA) are used.⁵ RF has been associated with several hormonal interferences leading to erroneous high levels of thyrotropin,^{8,9} free thyroxine,¹⁰ gonadotropins (FSH, LH, HCG), and prolactin.⁹ Recently, a single center study performed in 437 consecutive patients with incidentally discovered adrenal adenomas, a non-suppressed ACTH concentration (> 20 pg/ml), and a non-suppressed cortisol concentration after 1 mg overnight dexamethasone suppression test (> 1.8 mcg/dl) identified 4 patients with antibody interference in ACTH immunoassay. In one of them RF was responsible for the interference.¹

Analytical interference associated with Immulite 2000 ACTH assay might be likely due to the use of two different

antibodies for ACTH quantification; one of them, a murine monoclonal anti-ACTH antibody and, the other one, a rabbit polyclonal anti-ACTH antibody that could react with serum RF or other unknown antibodies; however, Liaison ACTH immunoassay uses two monoclonal anti-ACTH antibodies which improve their specificity.

In conclusion, the interference in ACTH immunoassay due to the presence of RF or other autoantibodies in plasma can be unmasked with the use of other analyzers avoiding inappropriate clinical decisions. In the case of an unexpected analytical result a good collaboration between clinical and laboratory professionals is essential to rule out the possibility of any interference in the analytical technique.

Ethical approval

I confirm that we have obtained written informed consent of the patients.

Contributorship

Biochemical analysis: AGC and LJ. Interpretation of data: all authors. Manuscript writing: PI & JJD. All authors reviewed and edited the manuscript and approved the final version of the manuscript.

Declaration of competing interests

None.

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