Letter to the Editor

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Cortisol measurement challenges in managing ectopic Cushing's syndrome with etomidate

https://doi.org/10.1515/cclm-2025-1211
Received September 16, 2025; accepted December 2, 2025; published online December 15, 2025

To the Editor,

Ectopic Cushing's syndrome is a rare and complex condition resulting from an ACTH-secreting (or, rarely, CRH-secreting) tumor outside the pituitary, often associated with significant morbidity. Patients with this disorder require treatments to rapidly control cortisol excess [1–3]. Etomidate infusion is a fast-acting medical therapy that can sometimes normalize cortisol levels within a few hours [3]. Close monitoring of plasma cortisol is mandatory to avoid driving these patients into adrenal insufficiency. Therefore, immunoassay interference could be a potential pitfall that needs to be managed [4].

We report here the case of a 23-year-old man with ectopic Cushing's syndrome caused by a metastatic large cell neuroendocrine carcinoma (LCNEC) of the thymus. The patient presented with severe hypertension, oedema, and metabolic disturbances, needing fast pharmacological management before surgery. Endocrinological investigations identified a markedly elevated cortisol level of 2,114 nmol/L (reference range 102–535 nmol/L) associated with a plasma adrenocorticotropic hormone (ACTH) level of 786 ng/L (reference range 3.8–60.5 ng/L). Urinary (24 h) and midnight salivary cortisol were also elevated at 20,692 µg/24 h (reference range 3.5–45.0 µg/24 h) and 166.4 µg/L (reference range<1.0 µg/L) respectively. Blood aldosterone and renin measurements (after a resting period) were 208 ng/L (reference range<75 ng/L) and 0.10 ng/ml/h (reference range 0.20–1.40 ng/ml/h) respectively, giving an aldosterone/renin ratio of 208 (reference range<20).

Initial treatment with oral ketoconazole failed to control cortisol levels, leading to the use of intravenous etomidate. Etomidate is a synthetic carboxylated imidazole developed as an intravenous hypnotic induction anesthetic agent by its action on γ-aminobutyric acid type A (GABA-A) receptors in the central nervous system (CNS) [5]. It also inhibits the mitochrondial cytochrome p450-dependent adrenal 11β-hydroxylase, a key enzyme in the production of cortisol from its immediate precursor, 11-deoxycortisol, reducing cortisol levels within 12–24 h of infusion [5, 6]. This side effect of adrenal suppression makes it a useful therapy for severe hypercortisolism.

In our patient, cortisol was measured approximately every 6 h after etomidate initiation to achieve a target of 600–800 nmol/L. Serum cortisol levels improved rapidly to a nadir of 329 nmol/L within a few hours, requiring a transient supplementation with intravenous hydrocortisone. As the use of inhibitors of adrenal steroidogenesis may overestimate cortisol levels due to cross-reactivity with accumulated precursors, particularly 11-deoxycortisol, cortisol measurements were also performed by liquid chromatography coupled to mass spectrometry, available in our hospital clinical laboratory [4]. Figure 1 illustrates the serum cortisol measurements during etomidate infusion, using two different cortisol assays, along with mass spectrometry measurements of 11-deoxycortisol.

Most clinical laboratories routinely quantify serum cortisol by immunoassay. A wide range of methods using anti-cortisol antibodies are commercially available. It is well-documented that the reliability of these immunoassays can be compromised in cases of altered steroid metabolism, where structurally similar metabolites may interfere with the test antibodies. For this reason, manufacturers of commercially marketed steroid hormone immunoassays are required to test and provide a list of interfering substances, ideally

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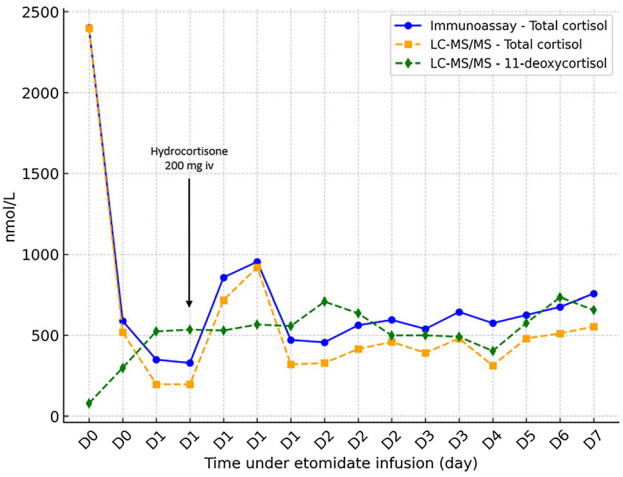

Figure 1: Serum cortisol and 11-deoxycortisol measurements during etomidate infusion.

accompanied by their cross-reactivity percentages, in the assay's package insert [4]. Although the specific interfering steroids and the degree of reactivity depend on the specific immunoassay used, the major cross-reacting compounds identified in cortisol immunoassays are cortisone, hydroxycortisone, prednisolone and 11-deoxycortisol. In our hospital laboratory, serum cortisol is routinely measured using a chemiluminescence microparticle competitive immunoassay (CMIA) on Alinity® (Cortisol CE-IVD Reagent Kit, Abbott, Chicago, IL, USA). Only pediatric cases and those requiring further investigation are analyzed by ultra-performance liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS) (Sciex Triple Quad 6500+ system, Marlborough, MA, USA). We use a method commercialised by ChromSystems (MassChrom® Steroids CE-IVD, ChromSystems, Munich, Germany) that measures a panel of 15 steroid hormones, including cortisol and its precursors, in serum in the same run. The LC-MS/MS technique offers enhanced sensitivity and specificity, along with the ability to separate and quantify multiple steroid hormones from a single sample in one analysis. However, LC-MS/MS is time-consuming and requires specialized personnel for sample preparation, mass spectrometer operation and result interpretation. While the two methods typically show good correlation, we have observed that serum cortisol levels of our patients measured by immunoassay tended to be higher compared to those measured by mass spectrometry. This discrepancy may result from etomidate's action on the steroidogenesis pathway resulting in the accumulation of 11-deoxycortisol. According to the immunoassay kit insert, Abbott reported a 1.9 % cross-reactivity for 11-deoxycortisol. Based on our findings, the interference appeared to be significantly higher, with a calculated median cross-reactivity of 26 %. This result is consistent with other studies showing biases ranging from 23 to 33.6 %. Monaghan et al. reported a 23 % cross-reactivity with 11-deoxycortisol on the Siemens ADVIA Centaur XP analyzer whereas the assay booklet listed a percentage of 7.3 % [7]. Similarly, Owen et al. observed a 28 % bias when using a Roche electrochemiluminescent immunassay as opposed to the 4.1 % mentioned by the manufacturer [8]. Additional data have also been collected on radioimmunoassays (RIA). Brossaud et al. found a cross-reactivity of 33.6 % with 11-deoxycortisol using a GammaCoat CA 1529 RIA kit (DiaSorin Corporation) vs. the 6.3 % indicated in the insert, and 28.3 % using a Spectria RIA kit (Orion Diagnostic), where the insert claimed just 0.3 % [9]. It is important to highlight that the methodology used to determine cross-reactivity differs between manufacturers and clinical studies. When available, the manufacturers' documentation indicated that cross-reactivity is commonly assessed using a controlled experimental approach in which the potential interferent is added to a steroid-free or stripped matrix, and parallelism is evaluated using the same immunometric method. In addition, accuracy and cross-reactivity are inherently dependent on the specificity of the antibodies used, which varies among commercial assays. Several hypotheses can therefore be formulated to explain the inconsistencies between the manufacturer-reported test values and those observed in clinical practice. Firstly, interfering molecules are evaluated at supra-physiological concentrations that do not reflect clinical reality [8]. Secondly, there is a possibility that steroid precursors in the biosynthesis pathway of cortisol, in addition to 11-deoxycortisol, may have a combined impact on the cross-reactivity observed in cortisol immunoassays [7]. The possibility of a matrix effect influencing the analysis results can also be considered. Additionally, this lack of consistency between test solutions and patient samples may overlook the equilibrium that exists *in vivo* between hormones and their binding proteins. These factors contribute to the wide variation in the observed data, which cannot therefore be extrapolated from one immunoassay to another, or from one patient to another. In our patient, the degree of over-estimation ranged from 6 to 65 %. This inter-assay, inter- and intra-individual variability makes it challenging to establish a consistent correction factor for cortisol values measured by immunoassays in patients receiving steroidogenesis inhibitors. This issue may be circumvented by the increasing availability of LC-MS/MS assays for steroid hormones, making it the preferred method for cortisol measurement to prevent potential erroneous clinical decisions related to dose adjustments of etomidate therapy.

In conclusion, this case report underscores the challenges in diagnosing and managing Cushing's syndrome associated with neuroendocrine thymic tumors. Additionally, it highlights the importance of recognizing the cross-reactivity of 11-deoxycortisol in cortisol immunoassays, emphasizing the need for mass spectrometry monitoring in patients treated with steroid biosynthesis inhibitors, such as etomidate.

Acknowledgments: The authors wish to thank Dr. Stefan Matei Constantinescu and Prof. Orsalia Alexopoulou from UCLouvain for sharing their expertise in the use of etomidate infusion.

Research ethics: Not applicable.

Informed consent: Not applicable.

Author contributions: All authors have accepted responsibility for the entire content of this manuscript and approved its submission.

Use of Large Language Models, AI and Machine Learning Tools: AI (ChatGPT and Deepl Write) was used to improve language.

Conflict of interest: The authors state no conflict of interest.

Research funding: None declared.

Data availability: Not applicable.

References

1. Reincke M, Fleseriu M. Cushing syndrome: a review. JAMA 2023;330:170–81.
2. Raff H, Carroll T. Cushing's syndrome: from physiological principles to diagnosis and clinical care. J Physiol 2015;593:493–506.
3. Constantinescu SM, Driessens N, Lefebvre A, Furnica RM, Corvilain B, Maiter D. Etomidate infusion at low doses is an effective and safe treatment for severe Cushing's syndrome outside intensive care. Eur J Endocrinol 2020;183:161–7.
4. Krasowski MD, Drees D, Morris CS, Maakestad J, Blau JL, Ekins S. Cross-reactivity of steroid hormone immunoassays: clinical significance and two-dimensional molecular similarity prediction. BMC Clin Pathol 2014;14:33.
5. Preda VA, Sen J, Karavitaki N, Grossman AB. Therapy in endocrine disease: etomidate in the management of hypercortisolaemia in Cushing's syndrome: a review. Eur J Endocrinol 2012;167:137–43.
6. Carroll TB, Peppard WJ, Herrmann DJ, Javorsky BR, Wang TS, Patel H, et al. Continuous etomidate infusion for the management of severe Cushing syndrome: validation of a standard protocol. J Endocr Soc 2019;3:1–12.
7. Monaghan PJ, Owen LJ, Trainer PJ, Brabant G, Keevil BG, Darby D. Comparison of serum cortisol measurement by immunoassay and liquid chromatography-tandem mass spectrometry in patients receiving the 11 β-hydroxylase inhibitor metyrapone. Ann Clin Biochem 2011;48:441–6.
8. Owen LJ, Halsall DJ, Keevil BG. Cortisol measurement in patients receiving metyrapone therapy. Ann Clin Biochem 2010;47:573–5.
9. Brossaud J, Barat P, Gualde D, Corcuff JB. Cross reactions elicited by serum 17-OH progesterone and 11-desoxycortisol in cortisol assays. Clin Chim Acta 2009;407:72–4.