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Case Report

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Falsely elevated serum oestradiol due to exemestane therapy

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Abstract

In this study, we present a case of falsely elevated oestradiol (E_2) concentration, determined by two immunoassays, in a breast cancer patient receiving exemestane therapy. The positive bias of immunochemical measurements was revealed using liquid chromatography tandem mass spectrometry which showed undetectable E_2 concentration. The discrepancy is expected to be a consequence of the structural resemblance of E_2 and exemestane sharing the same steroidal backbone. Inaccurate laboratory findings in therapy monitoring, as in this case, may lead to unnecessary changes of therapy.

Keywords

Immunoassay, mass spectrometry, steroid hormones

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Serum oestradiol (E_2) analysis plays an important role in the clinical investigation of postmenopausal women diagnosed with cancers which grow depending on oestrogen production. In such women, oestrogens are synthesized from androstenedione and testosterone. This reaction is catalysed by aromatase and primarily takes place in subcutaneous fat.[1,2] Aromatase enzyme inhibition using aromatase inhibitors (AIs) blocks this reaction and consequently either reduces the oestrogen's production or the oestrogen effect on the receptors.[3] Current AIs are divided into two subtypes: steroidal and non-steroidal. Steroidal AIs, such as exemestane, have a steroid-like structure similar to the aromatase substrate and bind to the aromatase enzyme's substrate-binding site. The enzyme converts exemestane into a reactive intermediate that covalently binds to the enzyme and causes its irreversible inhibition. Non-steroidal AIs, including anastrazole, bind non-covalently to the haem moiety of the aromatase enzyme and prevent androgen binding by saturating the binding-site. Since the main source of oestrogens in postmenopausal women arises from various steroid precursors which are aromatized in peripheral tissue, aromatase enzyme inhibition results in significant oestrogen reduction.[4]

The outcome of AI treated breast cancer mainly depends on the degree of E_2 suppression. E_2 concentration is expected to be very low, generally less than

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10 pmol/L.[5] It turns out that E_2 determination is critical for the selection of therapeutic approach. A lack of response to AI therapy suggests the need for therapy changes.

However, the determination of steroid hormones and E_2, in particular by immunoassay, is known to be prone to a positive bias caused by the existence of structurally related compounds.[6] In a patient receiving AI therapy, such an analytical method application may falsely indicate that the treatment goal has not been achieved. Therefore, in such instances, a more selective and a very sensitive method is required. In most laboratories, E_2 measurements are performed by direct immunoassays, using kits provided by various manufactures. These methods are easily available, fast and easy to apply and require small sample volumes, but are not specific enough for accurate E_2 determination and are also susceptible to different types of interferences. In contrast to immunoassays, liquid chromatography tandem mass spectrometry (LC-MS/MS) has increasingly become the method of choice in the area of endocrinology, particularly in steroid hormone measurements, because of its high specificity and sensitivity, wide dynamic range and the possibility to measure multiple analytes in only one analytical run.[5,7–9] However, the appropriate instrumentation is expensive and not widely available.

We herein report, according to our knowledge, the first case of exemestane interference, in a patient undergoing AI treatment in both Abbott and Roche, E_2 immunoassays.

Case

A 56-year-old woman, with a history of breast cancer, was examined for E_2 concentration. The breast cancer diagnosis was established in 2013. According to the tumour-nodes-metastasis classification of malignant tumours, she had T1N0M0 breast cancer and was subjected to a partial mastectomy and tamoxifen therapy. Tamoxifen, a drug used for the prevention of breast cancer relapse, is a selective oestrogen-receptor modulator. However, it shows adverse partial oestrogenic effect in the uterus and vascular system causing an increased risk of endometrial cancer and thromboembolism.[4] The control clinical imaging examination, which was performed a few months after the breast cancer diagnosis, revealed an abnormally thickened endometrium (10–12 mm) in the patient, which led to the replacement of the current therapy at the beginning of 2014. The new therapy, involving a non-steroidal AI, anastrazole, proved to be effective. Periodic serum E_2 assessments, measured by chemiluminescent microparticle immunoassay (CMIA) using an ARCHITECT i1000SR (Abbott Diagnostics, Lake Forest, USA) analyzer, were within the expected range (<37 pmol/L). Unfortunately, the patient developed joint pain as a side-effect of the anastrazole therapy, which was replaced at the beginning of 2015 by another AI, exemestane (25 mg). No side-effects have been reported since the introduction of the exemestane therapy. The determination of serum E_2 concentration, being a part of the patient's therapeutic monitoring, was requested in February 2016. E_2 concentration, measured by the above-mentioned CMIA method, was 854 pmol/L. Since the gynaecological ultrasound finding was normal, and the E_2 value was inconsistent with the clinical evidence, additional testing was suggested so as to evaluate the result. The patient was very concerned, considering that an elevated E_2 value would indicate the need for a total abdominal hysterectomy with bilateral salpingo-oophorectomy. She underwent venipuncture again. The laboratory findings of serum follicle-stimulating hormone and luteinizing hormone were as follows: 34.8 IU/L (reference interval for postmenopausal woman: 26.7–133.4 IU/L) and 10.37 mIU/L (reference interval for postmenopausal woman: 10.4–64.4 mIU/L). In addition, laboratory data showed a moderate decrease in serum prolactin concentration and an increased E_2 concentration, being 77.5 mIU/L (reference interval for woman over 21 years: 108.8–557.1 mIU/L) and 124 pmol/L (expected therapeutic value: <37 pmol/L), respectively. All of the above values were determined by the CMIA method implemented on an ARCHITECT analyzer. A blood sample was collected in a blood activator tube (Becton, Dickinson and Company, Franklin Lakes, NJ, USA) after a 20-min resting period. The sample was centrifuged for 10 min at 3000 r/min. The results of repeated measurements of E_2 were as follows: 140 pmol/L, 38 pmol/L, 45 pmol/L, 69 pmol/L and 87 pmol/L. Since the results were unexpectedly high and non-reproducible, two aliquots of the serum sample were frozen and stored at −20°C until conducting measurements using different methods. One aliquot was sent to the Department of Laboratory and Transfusion Medicine at Vukovar County General Hospital, Vukovar, Croatia, where the E_2 measurement was performed using an electro-chemiluminescence immunoassay method applied on a COBAS E411 analyzer (Roche Diagnostics, Indianapolis, USA). The measured E_2 values were: 87.2 pmol/L, 89.4 pmol/L and 91.1 pmol/L. The second aliquot was sent to the Institute of Laboratory Medicine, Clinical Chemistry and Molecular Diagnostics at Leipzig University Hospital, Leipzig, Germany, where the E_2 was determined by an LC-MS/MS, according to previously published[10] validated method with some modification. In brief, sample preparation included protein precipitation and online solid phase extraction. Analytic system

consisted of Shimadzu Prominence UPLC (Shimadzu, Diusburg, Germany) and QTRAP® 6500 mass spectrometer (SCIEX, Framingham, MA, USA). Electrospray ionization was applied in negative mode. The observed mass transitions in multiple reaction monitoring mode was 271/145 for E_2. Detailed method description is given in supporting material. The obtained E_2 value was less than the limit of quantification (<37 pmol/L). This value was in accordance with the result of the patient's clinical examination and effective therapeutic response.

Discussion

In this case, unexpectedly, high E_2 values were obtained by two different immunoassays. These values were inconsistent with the patient's clinical status and raised a suspicion about the presence of sample interferences. This suspicion was confirmed by E_2 determination using a more specific analytical method, namely the LC-MS/MS. Due to the considerable structural similarities of exemestane and E_2, the primary suspect was cross-reactivity of the drug. However, the manufacturer's package insert gave no indication of potential interferences by exemestane. Nevertheless, in April 2016, Abbott had sent an urgent field safety notice to all customers, in which they confirmed that the drug fulvestrant (Faslodex®) may interfere with the Architect E_2 assay leading to falsely elevated E_2 values. Both Siemens and Roche have issued field safety notice highlighting the same problem.[11] Fulvestrant is an oestrogen-receptor antagonist, also intended for the treatment of breast cancer in postmenopausal women. Due to the structural similarities between exemestane and fulvestrant, in this case, we substantiated our suspicion about falsely elevated E_2 due to cross-reactivity.

Cross-reactivity is a problem frequently associated with immunoassays, especially direct immunoassays without sample pretreatment step.[3] Matrix components and target molecules may be characterized by similar structures or cross-reactive epitopes. According to this, a positive bias may be the result of matrix components binding to the assay antibody.[6] Besides, such methods are not sensitive enough to measure the expected low E_2 concentrations presenting in postmenopausal women and those under AI treatment: high sensitivity assays and purification of E_2 from the plasma are required in those cases for trustworthy E_2 monitoring. Significance of accurate measurement of the oestrogens was recently discussed and some recommendations have been published.[12]

So far, only few studies dealing with the possible interference of exemestane in E_2 determination by immunochemical methods have been published. Suspicion regarding exemestane interference in E_2 radioimmunoassay has been raised by Johannessen et al.[13,14] as early as 1997. A study by Jacque et al.[3] included only three patients using exemestane therapy, among other patients taking different anticancer treatments. The presented data are insufficient for the establishment of a reliable conclusion concerning possible exemestane interference in E_2 immunoassays. Recently published data, obtained by Krasowski et al.[15] indicated that AIs, including exemestane, do not cause any detectable cross-reaction in spiked samples measured using the Roche E_2 kit for Elecsys and Modular E170 analyzers. Contrary to this, the results presented in this case report show a positive bias when using the Roche platform. One possible explanation for such inconsistent results may rest with exemestane metabolites, which represents a critical difference between matrices of patient and spiked samples. Another explanation may rely on different E_2 and exemestane concentrations. Unfortunately, Krasowsky et al. did not state explicitly which E_2 and exemestane concentrations they used. However, the question if oestradiol concentrations should be monitored in patients undergoing AI therapy for breast cancer has been recently debated.[16]

In conclusion, this case shows that exemestane may cross-react with Abbott Architect and Roche Cobas immunoassays for E_2. It presents yet further proof that clinicians should avoid reaching medical decisions on the basis of a single laboratory report, without taking into account the entire medical history of the patient, clinical examination and other findings. This is, especially the case in situations where the obtained results are inconsistent with other clinical data. Clinicians, clinical biochemists and reagent manufacturers should, at the very least, be aware of exemestane interference in the above mentioned E_2 immunoassays. This interference could lead to a false result and, subsequently, an improper diagnosis, an unsound medical decision and unjustified treatments. For appropriate clinical assessments, patients receiving exemestane should be monitored by the LC-MS/MS method.

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Ethical approval

The patient has given written informed consent for publication. The case report was approved by the Ethics Committee of the University Hospital Centre Osijek and it has been done in accordance with the Declaration of Helsinki.

Guarantor

SM.

Contributorship

SM conceived the study, wrote the manuscript and is responsible for the concept and design, data collection and interpretation. JK conducted oestradiol determination by LC-MS/MS, participated in results interpretation and manuscript preparation. DM contributed to the manuscript writing and reviewed the relevant literature. ZD assisted in data collection and provided critical insights. IL assisted in data collection and reviewed the relevant literature. VH assisted in data collection and was involved in gaining ethical approval. AG conducted oestradiol determination by LC-MS/MS. VS assisted in data collection. All authors reviewed and edited the manuscript and approved of its final version.

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