Interference in Estradiol assays from Hormone Therapies used in Breast Cancer Treatment

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Introduction

Two commonly used hormonal therapies in the treatment of estrogen-receptor positive breast cancers are Fulvestrant and Exemestane. Fulvestrant is an estrogen receptor antagonist, whilst Exemestane is an aromatase inhibitor. There is evidence that both Fulvestrant and Exemestane interfere with measurement of estradiol by immunoassay methods due to structural similarities. Most manufacturers of estradiol immunoassays do state in their accompanying literature that Fulvestrant interferes with measurement of estradiol, and advise users not to use their methods to measure estradiol in patients on Fulvestrant. Some state that aromatase inhibitors such as Exemestane may interfere. The majority of laboratories measuring estradiol do so by immunoassay. In any hospital serving a general, adult population and providing cancer care, laboratories are highly likely to analyse samples from patients on such hormonal cancer therapies.

The UK NEQAS for Steroid Hormones EQA scheme has almost 300 participants registered to receive specimens for assessment of estradiol measurement. Due to this, Birmingham Quality were well placed to investigate the impact of the presence of Fulvestrant and Exemestane on different manufacturers' estradiol immunoassays, and also to gather data on how aware laboratories are of the limitations of their estradiol assays in patients on hormonal cancer therapies.

Methods

Three pools of human off the clot serum from female donors were prepared that shared the same base pool and distributed at Distribution 523 of the UK NEQAS for Steroid Hormones EQA scheme (September 2024) as Specimens 523A, 523B and 523C respectively. Specimen 523A was the base serum with no added analytes. Specimen 523B contained 25 ng/mL Fulvestrant, based on the steady state concentrations stated in the literature (Brito et al., 2023, Endocrine Connections). Specimen 523C contained 150 pg/mL Exemestane, as an approximate plasma concentration at 24 hours' post dose stated in the literature (Valle et al., 2004, Br J Clin Pharmacol). Participants were required to analyse the specimens for estradiol. Percentage cross-reactivies of the results obtained from participants using each method with respect to the base pool result for that method were calculated for both specimens 523B and 523C. Participants were also asked a series of questions relating to how they handle estradiol requests in patients on hormonal cancer therapies including whether they vet or comment on requests, and if they refer any specimens for analysis by a mass spectrometry method.

Results

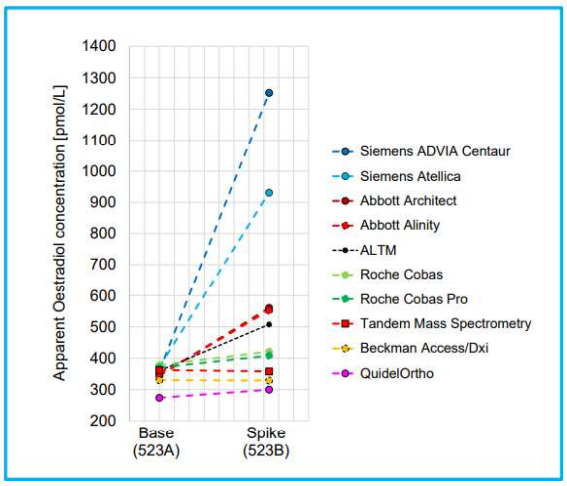

Figure 1: Absolute change in estradiol concentration in specimens spiked with 25 ng/mL Fulvestrant compared to the base pool for different estradiol methods within the UK NEQAS for Steroid Hormones EQA scheme.

Calculated cross-reactivities and percentage change in estradiol concentration from the base pool for each method for Fulvestrant and Exemestane are shown in Table 1. At the estradiol concentration of ~360 pmol/L and with 150 pg/mL Exemestane present, the percentage change in results for all methods was negligible.

Significant increases in measured estradiol concentration were observed when Fulvestrant was present, especially in the Siemens ADVIA Centaur and Atellica methods (Figure 1). A large increase was also observed in the Abbott Alinity and Architect methods, though this was not as large as in the Siemens assays. In all other methods, the percentage increase was negligible, and reassuringly did not change in the mass spectrometry method group.

The response rate to the questions and answers was 56% of registered participants in the scheme. With regard to whether participants analyse all requests for estradiol measurement or vetted them on the basis of clinical details, 85% of respondents analysed all specimens, with only 4% vetting requests.

When asked if participants refer any estradiol requests for analysis by mass spectrometry, 60% of respondents did not refer any specimens. Of the 42 participants that refer requests, there was a fairly equal split between those who refer at the request of the clinician (52%) and those who referred in specific clinical circumstances, including in Oncology patients on hormone therapies (48%).

Participants were asked if they comment on estradiol results about the possibility of interference in measurement of estradiol from some medications. Of the 106 respondents that answered the question, 59% did not append any comments to results regarding assay interference. Of the remaining 41%, 22% added comments manually at clinical review, and 17% added comments automatically.

25 ng/mL Fulvestrant	n	Base	Spike	Absolute Difference S - B	% cross reactivity	% change
ALTM	258	360	507	147	0.36	40.8
Abbott Alinity	52	346	552	206	0.50	59.5
Abbott Architect	14	344	560	216	0.52	62.8
Beckman Access/Dxi	29	330	329	-1	0.00	-0.30
QuidelOrtho	7	274	300	26	0.06	9.5
Roche Cobas Pro	8	370	408	38	0.09	10.3
Roche Cobas	90	378	421	43	0.10	11.4
Siemens ADVIA Centaur	13	359	1252	893	2.17	248.8
Siemens Atellica	27	372	931	559	1.36	150.3
Tandem Mass Spectrometry	16	362	358	-4	-0.01	-1.1

Table 1: Data displaying the difference between the base pool and pool spiked with 25 ng/mL as an absolute difference, percentage difference, and percentage cross-reactivity. It is important to note that cross-reactivity refers to the amount of Fulvestrant within the specimen that has been measured as estradiol by each assay at a Fulvestrant concentration of 25 ng/mL. The absolute difference would be the same regardless of the concentration of estradiol in the base pool.

Conclusions

The impact of Exemestane on different manufacturers' estradiol immunoassays was negligible. However, the concentration of 150 pg/mL Exemestane added to the specimens was chosen as an approximate concentration at 24 hours' post dose but this may have been too low.

Fulvestrant is a significant interferent in the measurement of estradiol by some manufacturers' immunoassay methods. The likely clinical impact of this is the over-estimation of the actual estradiol concentration in this patient group, potentially leading to inappropriate treatment decisions. It is reassuring that all manufacturers except QuidelOrtho, whose assay was not really impacted, clearly state in their accompanying literature that their assays should not be used to measure estradiol concentration in patients on Fulvestrant.

However, the results of the questions and answers suggest that many laboratories have not acted on all the supplied manufacturer information, or may not even be aware of the impact of Fulvestrant interference on their assay.