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LC-MS/MS detection of increased Androstenedione levels in patients receiving Danazol therapy

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To the Editor

Danazol is a 17-ethinyl testosterone derivative (1). It has multiple levels of action, which include suppression of pituitary gonadotropin secretion, direct inhibition of endometriotic implant growth, and direct inhibition of ovarian enzymes responsible for estrogen production (1). Danazol has long been used in the management of endometriosis, however its reported immunomodulatory effects such as reducing interleukin-1 and TNF- α have led to the use of danazol in management of immune related conditions such as idiopathic thrombocytopenic purpura (ITP) and aplastic anemia(1,2,3,4) . The side effects associated with danazol are largely due to its androgenic effects (1). Danazol has been reported to act as an interference in the immunoassay measurement of various androgens (including androstenedione), resulting in falsely elevated values for these hormones (5). As a consequence measurement of these hormones in patients receiving danazol therapy is best performed by liquid chromatography tandem mass spectrometry (LC-MS/MS).

Here we report eight cases of significantly elevated androstendione (AND) levels following LC-MS/MS measurement in patients receiving danazol therapy for aplastic anemia. Increased androstenedione values were identified at either 6 or 12 month follow- up post Danazol initiation (see Table 1a & 1b below for summary of the relevant laboratory

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DECLARATIONS

Conflicts of interest: None to declare

investigations at baseline and at follow-up). Whilst on therapy patients responded well and side effects of therapy were reported to be minimal.

LC-MS/MS measurement

Androstenedione is measured as part of a steroid hormone panel. Samples were prepared as per previously published method (6,7). An Agilent 6460 triple quadrupole mass spectrometer (Agilent, USA) equipped with an atmospheric pressure photoionization was used, employing isotope dilution with deuterium labeled internal standard for each analyte. Quantitation by multiple reaction monitoring (MRM) was performed in the positive ion mode. The quantifier MRM transition for androstenedione used was 287.2 > 97.1 and the qualifier MRM of 287.2 > 109 was used to confirm. The ratio of response of the two MRMs used, ranged from 56-58%. Retention times (RT) for AND were 6.540 -6.556 minutes for patient samples and 6.529- 6.554 minutes for the accompanying internal standard (IS). The ratio of androstenedione RT to internal standard RT for each specimen run was 1.00, thus confirming the identity of the peak.

The common metabolites of danazol present in the serum namely, ethisterone and 2-hydroxymethyl- ethisterone (2-HME) have been reported to affect immunoassay measurements of androgens due to cross reactivity with assays or by affecting hormone binding protein concentrations (8). The molecular weight of each metabolite is 312.45 and 342.47 respectively and thus unlikely to interfere with LC –MS/MS measurement of androstenedione which has different MRM's.

One of the important advantages of mass spectrometry analysis is the greater specificity of mass spectrometry measurement over immunoassay based testing. In the above described cases. Use of two MRM transitions (quantifier and qualifier) enabled the laboratory to confirm the presence of elevated androstenedione and exclude the likelihood of an interference.

Conclusions

Danazol increased the concentrations of androstenedione in all 8 patients with all being elevated to levels >97.5th percentile. Similar increases were not observed for the other androgens measured. Two of the 3 males had significant estradiol levels after treatment while all the females studied had normal estradiol concentrations during therapy. This finding raises interesting questions as to the effect of danazol on estrogen concentrations.

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Table 1a

Summary of results for female patients (n=5)^a

Female Patients	Patient 1		Patient 2		Patient 3		Patient 4		Patient 5	
	Baseline	Follow-up	Baseline	Follow-up	Baseline	Follow-up	Baseline	Follow up	Baseline	Follow up
AND (ng/dL) LC-MS/MS	38 (30-200)	8128 (17-175)	26 (30-200)	33703 (17-175)	140 (30-200)	21374 (17-175)	176 (30-200)	29373 (17-175)	104 (30-200)	13299 (17-175)
17 OHP (ng/dL) LC-MS/MS	< 40 (< 220)	12 (13-175)	<40 (< 220)	<40 (< 220)	<40 (< 220)	<40 (< 220)	42 (< 220)	<40 (< 220)	101 (< 220)	<40 (< 220)
DHEA(ng/dL) LC-MS/MS	140 (<1300)	209 (0-599)	120 (<1300)	43 (0-599)	550 (<1300)	458 (0-599)	1500 (<1300)	829 (0-599)	300 (<1300)	208 (0-599)
Estradiol(pg/mL) LC-MS/MS		46.1 (12-460)		55.92 (12-460)		56.57 (12-460)		46.65 (12-460)		51.84 (12-460)
Estradiol(pg/mL) Immunoassay		186 (15-350)	36 (15-350)	261.3 (15-350)	78.9 (15-350)	149.1 (15-350)	46.1 (15-350)	184.4 (15-350)	117 (15-350)	151.8 (15-350)
SHBG(nmol/L) Immunoassay	166 (18-114)	10 (18-114)		14 (18-114)	21 (18-114)	3 (18-114)				15 (18-114)
TT (ng/dL) Immunoassay	20 (<81)	32.0 (< 81)	<20 (< 81)	32.2 (< 81)	<20 (< 81)	<20 (< 81)	27.2 (< 81)		<20 (< 81)	33.4 (< 81)

AND-androstenedione; 17 OHP- 17 hydroxyprogesterone; DHEA- Dehydroepiandrosterone ; TT- Total testosterone; SHBG- sex hormone binding globulin

^a Different reference intervals for the same assay occur where test was previously referred and later performed in-house; values in parantheses () are reference intervals

Table 1b

Summary of results for male patients (n=3)^a

Male patients	Patient 6		Patient 7		Patient 8	
	Baseline	Follow up	Baseline	Follow up	Baseline	Follow-up
AND (ng/dL) LC-MS/MS	105 (40-150)	14239 (25-125)	168 (40-150)	5609 (25-125)	240 (40-150)	17325 (25-125)
17 OHP (ng/dL) LC-MS/MS	61 (<220)	30 (13-120)	124 (<220)	44 (<220)	84 (<220)	<40 (<220)
DHEA (ng/dL) LC-MS/MS	860 (<1300)	159 (0-649)	520 (<1300)	706 (0-649)	440 (<1300)	214 (0-649)
Estradiol (pg/mL) LC-MS/MS		69.3 (8-33)		16.3 (8-33)		47.8 (8-33)
Estradiol (pg/mL) Immunoassay			39.2 (0-56)		83.2 (0-56)	163.7 (0-56)
SHBG (nmol/L) Immunoassay	55 (13-71)	15 (13-71)				
TT (ng/dL) Immunoassay	322 (262-1593)	105 (262-1593)	547 (262-1593)			

AND-androstenedione; 17 OHP- 17 hydroxyprogesterone; DHEA- Dehydroepiandrosterone ; TT- Total testosterone; SHBG- sex hormone binding globulin

^a Different reference intervals for the same assay occur where test was previously referred and later performed in-house; values in parantheses () are reference intervals