RESEARCH ARTICLE

Are serum cortisol measurements by immunoassays reliable?: A case series

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Abstract

Routinely used automated immunoassays have been found to give unrealiable measurements of thyroid hormones in the presence of either high or low levels of thyroxine-binding globulin. Thyroid hormones are not the only analytes bound to specific binding proteins that are measured by immunoassays. Preliminary data from a series of cases, comparing IA measurements to those obtained by liquid chromatography-tandem mass spectrometry, reveal for the first time that IA measurements report falsely low (by an average of 27%) serum cortisol concentrations. Initial findings suggest that IA measurements of serum cortisol are affected by high concentrations of corticosteroid binding globulin.

1. Introduction

Serum cortisol is routinely measured by automated immunoassay (IA) in the laboratory.[1] It is well known that direct IA measurements of serum cortisol suffer from interferences caused by cross-reactivity of structurally similar metabolites.[2–5] These interferences and their implications for clinical diagnoses have led to standardization initiatives, such as the Hormone Standardization Program (HoST)[6–8], which recommend the use of liquid chromatography-tandem mass spectrometry (LC-MS/MS) for all laboratory measurements of steroid hormones. Yet, the convenience and low-cost of automated IA often outweighs this recommendation. Finally, IAs are very precise but often give you precisely the wrong answer. Here, we describe the potential for yet another limitation of measuring serum cortisol concentration by IA, and the possible negative clinical implications that can occur by relying on IA measurements.

1.1 Limitations of IAs as a result of high or low concentrations of specific binding proteins

IAs have also been found to give unreliable measurements of free thyroxine (FT4) and free tri-iodothyronine (FT3) as a result of either high or low levels of thyroxine-binding globulin (TBG).[9–14] Like FT3 and FT4, cortisol also binds to its own specific binding protein, corticosteroid-binding globulin (CBG). Similar to TBG levels, CBG concentrations can be affected by critical illness. In addition to a handful of rare CBG-gene mutations, common illnesses such as preeclampsia, diabetes, renal and hepatic failure decrease CBG levels.[15] Increases in CBG concentrations can occur during pregnancy and oral estrogen administration.[12,16,17] So analytes, FT3, FT4 and cortisol present similar challenges for IA measurements. We hypothesize that alterations in CBG levels do affect the reliability of IA measurements of cortisol.

2. Discrepancy between IA and LC-MS/MS measurements of serum cortisol in patients with high CBG levels

As the first step in identifying whether IA measurements of serum cortisol are also unreliable as a result of high or low concentrations of CBG, we describe three cases in which significantly high levels of CBG correspond to falsely low IA measurements of cortisol when compared to those obtained by a reference LC-MS/MS method. Three reproductive age females (Table 1) taking the same combined oral contraceptive pill (OCP) (drosepirenone/ethinyl estradiol), presented with CBG concentrations that were far higher than the 97.5th percentile (Table 2). While hepatic CBG synthesis is known to increase in patients receiving estrogen therapy or taking high-dose oral contraceptives[18,19], we were surprised to see significantly lower cortisol levels by IA than by LC-MS/MS in these 3 cases. Since CBG levels were high, we might also expect the IAs to reflect higher, not lower, total cortisol levels. These results were also inconsistent with a previous study[20], in which the oral administration of combined OCPs containing ethinyl estradiol resulted in high CBG concentrations as well as high serum levels of IA-measured cortisol. Unfortunately the latter study did not compare IA with LC-MS/MS values.

Table 1. Clinical presentation highlights of three patients with unusual IA cortisol results taking the same combined OCP

	phenotype and pathophysiology	drospirenone/ethinyl estradiol (mg/day)
Case 1	A 33 year old African female with a history of polycystic ovarian syndrome (PCOS), obesity, cholelithiasis s/p laparoscopic cholecystectomy	3/0.3
Case 2	A 28 year old Caucasian female with a history of non-classic congenital adrenal hyperplasia (CAH) with long term treatment with prednisone 4 mg/day	3/0.02
Case 3	A 24 year old Caucasian female with a history of pleuropulmonary blastoma and pathogenic *DICER1* mutation (c. 1966C>T; autosomal dominant inheritance with decreased penetrance) with a maternal lineage history of cervical embryonal rhabdomyosarcoma	3/0.02

Table 2. Serum steroid profiles by IA and LC-MS/MS of cases 1–3 (samples drawn between 6–10 AM).

Analyte (reference range)	Case 1	Case 2	Case 3	Mean value
IA[a]				
cortisol (5–25 µg/dL)	15.7[a]	20.6[a]	23.7[a]	**19.7**
	13.7[b]	22.1[b]	22.3[b]	
Testosterone[b] (<20–80 ng/dL)	<20[b]	35.2[b]	<20[b]	
sex hormone binding globulin (SHBG)[b] (18–114 nmol/L)	**364**	106	**164**	
corticosteroid binding globulin (CBG)[c] (1.7–3.1 mg/dL)	**6.2**	**5.5**	**4.6**	
LC-MS/MS				
Cortisol[d] (6–10 AM: 6.5–34.9 µg/dL)[d]	23.1	28.2	29.0	**26.8**
Testosterone[d] (6–10 AM: 9–84.8 ng/dL)[d]	21.53	70.62	28.62	**40.2**

[a]Abbott Architect ci8200. [b]Siemens Immulite 2000 analyzer. [c]Esoterix endocrinology laboratory. [d]Diurnal steroid hormone reference ranges[21] used by the National Institutes of Health (NIH).

Discordance between high concentrations of CBG, and lower than expected cortisol concentrations led us to evaluate cortisol levels by a reference LC-MS/MS method[22,23]. In contrast to antibody based assays, structurally based assays like mass spectrometry are highly specific for the cortisol molecule.[22,23] Remarkably, serum cortisol measurements by LC-MS/MS (mean = 26.8 µg/dL) were on average 27% *higher* than those evaluated by two independent automated IA instruments (mean = 19.7 µg/dL) (Table 2). These results are very unusual since IA measurements of cortisol are susceptible to cross-reactivity with structurally similar compounds, typically leading to higher values when compared to LC-MS/MS.[1]

2.1 High concentrations of CBG affect the reliability of IA serum cortisol measurements, how will low concentrations of CBG affect serum cortisol measurements?

We suggest that high concentrations of binding proteins cause falsely low results in many cortisol IAs. This is not surprising as these proteins avidly bind to the analyte being measured and compete for the analyte with the antibody used in the IA. We have shown that this situation exists when measuring FT4 and FT3 at high and low concentrations of TBG.[9,10] These three patients show that high CBG concentrations may be the cause of the falsely low measurements by IA. However, further investigation is required to determine if cortisol measurements by IA are also unreliable in patients with low CBG levels.

2.2 How do these findings translate to the general clinical practice and future studies?

The use of analytical instruments and validated methodologies capable of accurate measurements is paramount. 64.9% of women between 15–49 years old are using oral contraceptives in the United States.[24] Therefore, cortisol measurements in these instances, or during pregnancy, should follow the most rigorous protocols (accounting for variations in binding proteins) to provide the right diagnosis to the right patient. Future studies will evaluate the influence of CBG concentrations on IA and LC-MS/MS serum cortisol measurements in a larger population of women between the ages of 15–49 taking OCPs, focusing on women with cortisol concentrations above 12 µg/dL.

2.3 Are IA measurements reliable for any analyte that can be affected by high or low levels of a specific binding protein?

When assessing testosterone by IA and LC-MS/MS, all three cases were found to have higher serum testosterone concentrations when measured by LC-MS/MS than by IA. Again, an abnormal finding! These results indicate that measurements of testosterone might also be affected by SHBG concentrations >97.5th percentile. Interestingly, these preliminary results demonstrate that high levels of SHBG do not necessarily correspond to high serum concentrations of testosterone.

3. Conclusion

Our preliminary findings raise questions about the reliability of IA measurements for *any* analyte that can be affected by high or low concentrations of a specific binding protein. In this study, the IA measurements of cortisol and testosterone reported falsely low values when CBG and SHBG concentrations were >97.5th percentile. This situation occurs frequently in women taking oral contraceptives. This study will be further validated. Future studies will also evaluate whether inconsistencies between IA and LC-MS/MS measurements occur for testosterone, cortisol and estradiol when low (<2.5th percentile) binding

protein concentrations are observed as in some patients with renal disease.

Funding

Dr. Soldin receives Intramural NIH Research funding.

Conflict of interest statement

The authors declare that there is no conflict of interest.

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