Peak Serum Cortisol Cutoffs to Diagnose Adrenal Insufficiency Across Different Cortisol Assays in Children

Cortez S et al. Cutoff Point to Diagnose Adrenal Insufficiency Using Cortisol Monoclonal Antibody Immunoassay

Introduction

Adrenal insufficiency (AI) is a common condition characterized by a deficient production of glucocorticoids. The correct diagnosis of AI is of utmost importance as it may be life-threatening if left untreated. However, the incorrect diagnosis of adrenal insufficiency can have a major negative impact on the patient and their family’s lives, including medication cost, ongoing medical care, and potential side effects and comorbidities from unnecessary treatment with corticosteroids.(1)

Conventionally, the biochemical diagnosis of AI is established through Cosyntropin stimulation test.(2) This test assesses the adequate cortisol response to stimulation with either a 250 mcg Cosyntropin when primary adrenal insufficiency is suspected or 1 mcg for evaluation of central adrenal insufficiency or when there is a Cosyntropin shortage. In both instances, a peak serum cortisol cutoff of less than 18 μg/dl (500 nmol/L), using a traditional polyclonal antibody (pAb) immunoassay is considered diagnostic of adrenal insufficiency.(3)

Consequently, the routine routine use of a peak cortisol cutoff of 18 μg/dl (500 nmol/L) can lead to over diagnosis of AI in children undergoing a Cosyntropin stimulation test due to the use of a polyclonal antibody (pAb) immunoassay. However, it has been suggested that newer and more specific cortisol monoclonal antibody immunoassays may lead to an over-diagnosis of AI in children.(4) The most commonly used immunoassay for measuring serum cortisol is the polyclonal antibody (pAb) immunoassay (Roche Elecsys Cortisol I), mAb immunoassay (Roche Elecsys Cortisol II), and LC/MS. Logistic regression was used to predict AI using the pAb as the reference standard. A receiver operator characteristic curve (ROC), area under the curve (AUC), sensitivity, specificity, and kappa agreement were also calculated.

Methods

Cortisol levels from 36 children undergoing 1 mcg Cosyntropin stimulation test to rule out AI were measured using polyclonal antibody (pAb) immunoassay (Roche Elecsys Cortisol I), mAb immunoassay (Roche Elecsys Cortisol II), and LC/MS. Logistic regression was used to predict AI using the pAb as the reference standard. A receiver operator characteristic curve (ROC), area under the curve (AUC), sensitivity, specificity, and kappa agreement were also calculated.

Results

Using a peak serum cortisol cutoff value of 12.5 μg/dL for the mAb immunoassay provides a 99% sensitivity and 94% specificity for diagnosing AI, when compared to the historical pAb immunoassay cutoff of 18 μg/dL (AUC= 0.997). Likewise, a cutoff of value of 14 μg/dl using the LC/MS, provides a 99% sensitivity and 86% specificity when compared to the pAb immunoassay (AUC=0.995).

Conclusion

To prevent overdiagnosis of AI in children undergoing 1 mcg Cosyntropin stimulation test, our data support using a new peak serum cortisol cutoff of 12.5 μg/dL and 14 μg/dL to diagnose adrenal insufficiency using pAb immunoassays and LC/MS in children, respectively.

Keywords: Adrenal insufficiency, cortisol, assays, pediatrics.
a pAb immunoassay to 71% when the mAb immunoassay was adopted. (9) Previous studies in adults, (10, 11, 12) report conflicting results when establishing a new cutoff level for the biochemical diagnosis of AI. These studies were conducted in adult population and using ACTH stimulation test with Cosyntropin 250 mcg. Some have proposed a new cortisol cutoff after Cosyntropin stimulation test of 14 to 15 µg/dl for mAb immunoassay and LC/MS. (10, 11) Others consider the new cutoff to be 12.7 µg/dl when using the mAb immunoassay. (12) Moreover, these studies do not provide any information about diagnostic accuracy for the proposed cutoff level or the agreement between the proposed cutoff and the clinically accepted cutoff value when using pAb immunoassay. In addition, there is paucity of data in the pediatric population and when using Cosyntropin 1 mcg for the stimulation test. Thus, the aim of this study is to establish the optimal peak serum cortisol cutoff when mAb immunoassay and LC/MS are used in pediatric patients undergoing 1 mcg Cosyntropin stimulation test. We will also determine the sensitivity and specificity of these cutoffs to diagnose AI, as well as the probability of agreement between the assays using a kappa statistic.

Methods
De-identified blood samples were prospectively collected from pediatric patients undergoing 1 mcg Cosyntropin stimulation test at St. Louis Children’s Hospital from July 1st, 2016, to July 31st, 2017. Samples were analyzed using pAb immunoassay (Roche Elecsys Cortisol I), mAb immunoassay (Roche Elecsys Cortisol II), and liquid chromatography tandem mass spectrometry (LC/MS), which is considered the reference standard.

For the pAb immunoassay, we used Roche Elecsys Cortisol I. The analysis was completed using Roche automated system (Cobas 601). This assay has a within-run precision of 1.6% coefficient variation (CV) at 3.6 µg/dL and 24.2 µg/dL. The between-run precision is 3.2% at 3.7 µg/dL and 1.8% at 24.1 µg/dL. All samples were analyzed at the same time under this assay.

For the mAb immunoassay, we used Roche Elecsys Cortisol II, which makes use of a competition test principle using a monoclonal antibody, which is specifically directed against cortisol. The analysis was completed using Roche automated system (Cobas 601). This assay has a within-run precision of 11.1% CV at 4.0 µg/dL and 22.9 µg/dL with a between-run precision of 2.4% CV at 4.1 µg/dL to 12.5 µg/dL. Under this assay, samples were analyzed on the day of collection over the study time.

The LC/MS assay was performed at Mayo Clinic Laboratory in Rochester, Minnesota. Deuterated cortisol (d4-cortisol) is added to each sample specimen with methyl chloride and analyzed by liquid chromatography-tandem mass spectrometry using multiple reaction monitoring. Assay has no crossreactivity, as previously published. (13)

Statistical Analysis
Peak serum cortisol level is used at each different assays and using mean peak serum cortisol level with standard deviation was calculated. Peak serum cortisol level is defined as the highest cortisol level at any point during stimulation test. Samples were collected at 20, 30, and 60 min after Cosyntropin is given. We defined adrenal insufficiency as a peak serum cortisol below 9.8 µg/dL or using the pAb immunoassay.

Measurements by LC/MS and mAb immunoassay were individually used in simple logistic regression models to predict adrenal insufficiency. For each model, we used receiver operator characteristic (ROC) curve, area under the curve (AUC), sensitivity, and specificity, to evaluate the potential of the median values as thresholds for each predictor. Also, kappa agreement statistic between the new cutoffs and the historic peak serum cortisol cutoff of 18 µg/dL when using a traditional pAb immunoassay was calculated.

Results
36 de-identified serum samples from pediatric patients undergoing 1 mcg Cosyntropin stimulation test were collected during the study and compared across all 3 laboratory assays.

The mean (±SD) serum cortisol level using the pAb immunoassay was 17.1 ± 8.1 µg/dL. While the mean (±SD) serum cortisol level using the mAb immunoassay was 12 ± 6.6 µg/dL. As shown in Figure 1, over 75% of all mAb values were below the historic cutoff of 18 µg/dL, meeting the biochemical diagnosis of adrenal insufficiency. The mean difference in serum cortisol level between the mAb immunoassay and the pAb immunoassay was 5.12 µg/dL (p-value < 0.001). The area under the ROC for the mAb immunoassay ROC was 0.997 (Figure 2). Using a cutoff of 12.5 µg/dL for the mAb immunoassay, provides a sensitivity of 99% (95% CI 99% – 100%) and specificity of 94% (95% CI 87% – 100%). Furthermore, a simple kappa agreement between the cutoff for the mAb immunoassay and the pAb immunoassay was calculated to be 0.94 (95% CI 0.88 – 1.00). The mean (±SD) serum cortisol level for the LC/MS assay was 12.9 ± 6.6 µg/dL. As presented in Figure 1, 75% of all the LC/MS values obtained were below the current threshold of 18 µg/dL, meeting the biochemical diagnosis of adrenal insufficiency. The mean difference in serum cortisol level between the LC/MS assay and the pAb immunoassay was 4.2 µg/dL (p-value <0.01) The AUC for the LC/MS ROC was 0.995 (figure 3).

Using a peak serum cortisol cutoff of 14 µg/dL when using LC/MS, provides a sensitivity of 99% (95% CI 96 – 100%) and specificity of 88% (95% CI 79% – 97%). A simple kappa agreement between the cutoff for the pAb immunoassay and the LC/MS assay was calculated to be 0.888 (95% CI 0.80 – 0.97).

Discussion
This study found that serum cortisol levels in children using mAb immunoassay and LC/MS were statistically and clinically significantly lower than the traditional pAb immunoassay, which in a clinical setting can potentially translate in an overdiagnosis of adrenal insufficiency and increase morbidity for the patient due to unnecessary use of steroids. Therefore, our data supports the need to redefine the biochemical peak serum cortisol cutoffs. We propose a new peak serum cortisol cutoff of 12.5 µg/dL and 14 µg/dL when using mAb immunoassay or LC/MS are used, respectively.

This is the first study to do a head to head comparison between the different cortisol assays in a pediatric population undergoing low dose Cosyntropin stimulation test for the biochemical diagnosis of adrenal insufficiency. Our monoclonal and LC/MS assay cutoffs demonstrated a high sensitivity and specificity to ensure that the diagnosis of adrenal insufficiency is not missed. We demonstrated a strong kappa correlation between the traditional peak cortisol level cutoff of 18 µg/dL for the diagnosis of adrenal insufficiency and the cutoffs proposed in the study (12.5 µg/dL for mAb immunoassay and 14 µg/dL for LC/MS), which to our knowledge have not been calculated in previous studies. These values information strengthens the rationale to redefine the cutoff for the diagnosis of adrenal insufficiency when using mAb immunoassay and LC/MS.

This study is unique for being performed entirely at a pediatric infusion center, under the same protocol and procedures. Keeping this variables constant adds rigor and reproducibility to the study design and demonstrate that the differences in cortisol levels are assay-specific, leading to different cutoff values that are reliable and applicable in a pediatric population. Moreover, all samples were analyzed using the pAb immunoassay, mAb immunoassay, and LC/MS, which is considered the reference standard when using 1 mcg Cosyntropin stimulation test in pediatric population. (14) Additionally, one possible limitation of this study is the use of 1 mcg Cosyntropin stimulation test and the potential variability in results compared to the 250 mcg Cosyntropin stimulation test. Nevertheless, recent studies determined that both 250 mcg and 1 mcg stimulation tests have similar diagnostic...
accuracy for diagnosing adrenal insufficiency, which is also supported by the similar results obtained between our study and previous ones. (15, 16).

Another limitation of this study is that the use of de-identified patient samples did not allow for individual patient or demographic analysis. However, as previously mentioned, the biochemical diagnosis of adrenal insufficiency is based on the clinically widely used criteria of peak cortisol levels lower than lower than 18 μg/dL (500 nmol/L) using a traditional pAb immunoassay. Future research is required to validate these proposed cutoff points using biochemical and clinical information. However, our results agree with previous studies demonstrating that newer and more specific mAb immunoassay yields lower serum cortisol values leading to the overdiagnosis of AI and unnecessary steroid use. Based on our results and previous studies, we recommend a new cutoff value of 12.5 μg/dL when using mAb immunoassay and 14 μg/dL when using LC/MS.

**Statements**

**Statement of Ethics**

Study was granted a waiver of written informed consent and data collection approved by the IRB/Human Research Protection Office at Washington University in Saint Louis (IRB ID # 202012130).

**Disclosure Statement**

The authors have no conflicts of interest to declare.

**Data Availability Statement**

All data generated or analyzed during this study are included in this article. Further inquiries can be directed to the corresponding author.

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**Author Contributions**

SC and KM contributed to the conception and design of the study, collected, analyzed, and interpreted data, and drafted the initial version of the manuscript. AMA contributed to the conception and design of this study, interpreted, and analyzed data, provided methodologic expertise, and critically reviewed and revised the manuscript for important intellectual content. MW provided statistical analysis expertise, collaborated with the analysis of data.

**References**

Figure 1. Peak cortisol level (mcg/dL) using polyclonal antibody immunoassay, monoclonal antibody immunoassay, and liquid chromatography mass spectrometry (LC/MS) in 36 children undergoing 1 mcg Cosyntropin stimulation test.
Figure 2. Receiver operating characteristic curve for the diagnosis of adrenal insufficiency based on the peak cortisol level during a 1 mcg Cosyntropin stimulation test measured by monoclonal antibody immunoassay (mAb)
Figure 3. Receiver operating characteristic curve for the diagnosis of adrenal insufficiency based on the peak cortisol level during a 1 mcg Cosyntropin stimulation test measured by liquid chromatography mass spectrometry (LCMS)