ORIGINAL ARTICLE

DOI: 10.4274/jcrpe.galenos.2023.2023-2-3

J Clin Res Pediatr Endocrinol 2023;15(4):375-379

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

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What is already known on this topic?

Previous reports suggest that newer and more specific cortisol assays result in lower cortisol values than the traditional polyclonal antibody (pAb) immunoassay. However, no specific peak serum cortisol cutoff value for the diagnosis of adrenal insufficiency (AI) in children has been established for these newer and more specific assays and no information about diagnostic accuracy has been provided.

What this study adds?

This study redefines the biochemical diagnostic cutoff points for AI in children when using a highly specific cortisol monoclonal antibody immunoassay and liquid chromatography tandem mass spectrometry. It also provides information on diagnostic accuracy for each cut off point when compared to the reference pAb immunoassay.

Abstract

Objective: Current peak serum cortisol cutoffs for the diagnosis of adrenal insufficiency (AI) after Cosyntropin stimulation have been established using polyclonal antibody (pAb) immunoassays. However, new and highly specific cortisol monoclonal antibody (mAb) immunoassays are being used more widely, which can potentially yield higher false positive rates. Thus, this study aimed to redefine the biochemical diagnostic cutoff points for AI in children when using a highly specific cortisol mAb immunoassay and liquid chromatography tandem mass spectrometry (LC/MS) to avoid unnecessary steroid use.

Methods: Cortisol levels from 36 children undergoing 1 mcg Cosyntropin stimulation tests to rule out AI were measured using 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, 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 provided 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, provided 99% sensitivity and 88% 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 AI when using mAb immunoassays and LC/MS in children, respectively.

Keywords: Adrenal insufficiency, cortisol, assays, pediatrics

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Conflict of interest: None declared

Received: 03.03.2023

Accepted: 09.05.2023

Introduction

Adrenal insufficiency (AI) is a common condition characterized by a deficient production of glucocorticoids. The correct diagnosis of AI is of the utmost importance as it may be life-threatening if left untreated. However, the incorrect diagnosis of AI 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 comorbidity from unnecessary treatment with corticosteroids (1).

Conventionally, the biochemical diagnosis of AI is established through Cosyntropin stimulation test (2). This test assesses the adequacy of cortisol response to stimulation with either a 250 mcg dose of Cosyntropin when primary AI is suspected or 1 mcg for evaluation of central AI 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 AI (3).

The pAb immunoassay lacks specificity with varying degrees of antibody cross-reactivity with endogenous proteins. Therefore, in many institutions, this assay has been replaced with other more specific laboratory assays; liquid chromatography tandem mass spectrometry (LC/MS) or monoclonal antibody (mAb) immunoassay. Though cortisol immunoassays are widely used for their high performance and cost-effectiveness (4), LC/MS is considered the gold standard test (5). Previous reports suggest that these newer assays result in lower cortisol values than the traditional pAb immunoassay (6). However, no specific peak serum cortisol cutoff value for the diagnosis of AI has been established for these newer and more specific assays (7,8).

The adoption of these newer cortisol assays while still using historic cortisol cutoffs may lead to an overdiagnosis of AI and unnecessary corticosteroid use. As demonstrated in a previous study, if the cortisol cutoff was not adjusted, the rate of AI diagnosis increased from 26% using 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 an adrenocorticotropic hormone stimulation test with Cosyntropin 250 mcg. Some researchers 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 was 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. Further aims were to 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 LC/MS, which is considered the reference standard.

For the pAb immunoassay, the Roche Elecsys Cortisol I was used and the analysis was completed using a Roche automated system (Cobas e601). This assay has a within-run precision of 1.6% coefficient of variation (CV) at 3.6 µg/dL and 24.2 µg/dL. The between-run precision is 3.0% at 3.7 µg/dL and 1.8% at 24.1 µg/dL levels. All samples were stored at -80 °C until batch analysis at the same time with this assay.

For the mAb immunoassay, the Roche Elecsys Cortisol II was used, which makes use of a competition test principle using a mAb, which is specifically directed against cortisol. The analysis was again completed using a Roche automated system (Cobas e601). The within-run precision is 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 and 2.5% CV at 23.3 µg/dL. With 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 specimen as an internal standard. Cortisol and d4-cortisol are extracted from the samples with methylene chloride and analyzed by LC/MS using multiple reaction monitoring. This assay does not suffer from crossreactivity, as previously published (13).

The study was approved by the Washington University in St. Louis of Institutional Review Board (IRB ID: 202012130, date: 08.09.2022).

Statistical Analysis

Peak serum cortisol level using each different assay was obtained and mean peak serum cortisol level with standard deviation (SD) was calculated. Peak serum cortisol level is defined as the highest cortisol level at any time point during the stimulation test. Samples were collected at 20, 30, and 60 min after Cosyntropin is given. We defined AI as a peak serum cortisol below 18 µg/dL using the pAb immunoassay.

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

Results

Thirty-six de-identified serum samples from pediatric patients undergoing 1 mcg Cosyntropin stimulation test were collected during the study and compared across the three different laboratory assays. The mean (±SD) serum cortisol level using the pAb immunoassay was 17.1±9.7 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 AI. The mean difference in serum cortisol level between the mAb immunoassay and the pAb immunoassay was 5.12 µg/dL ($p < 0.001$). The AUC for the mAb immunoassay ROC was 0.997 (Figure 2). Using a cutoff of 12.5 µg/dL for the mAb immunoassay provided a sensitivity of 99% (95% CI: 96-100%) 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 AI. The mean difference in serum cortisol level between the LC/MS assay and the pAb immunoassay was 4.2 µg/dL ($p < 0.01$) 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 provided 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: 0.800.97).

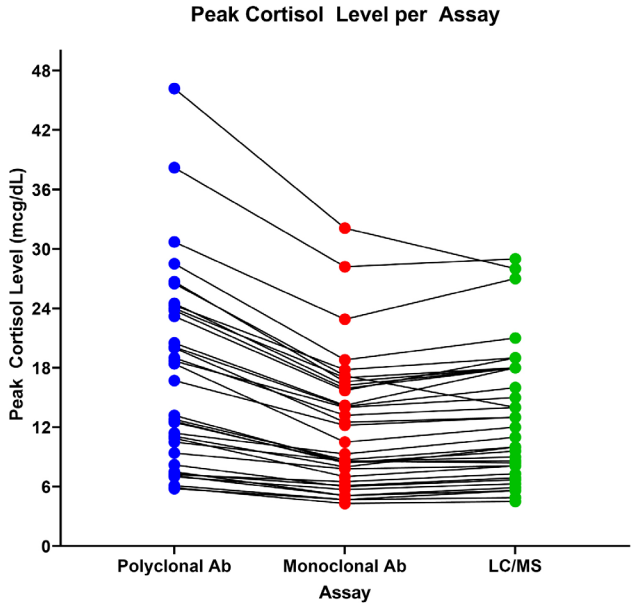

Figure 1. Peak cortisol level (mcg/dL) using polyclonal antibody immunoassay, monoclonal antibody immunoassay, and LC/MS in 36 children undergoing 1 mcg Cosyntropin stimulation test

LC/MS: liquid chromatography mass spectrometry

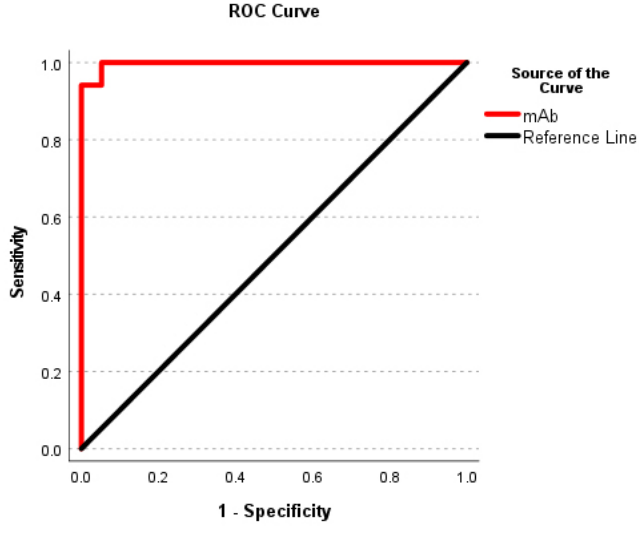

Figure 2. ROC curve for the diagnosis of adrenal insufficiency based on the peak cortisol level during a 1 mcg Cosyntropin stimulation test measured by mAb

mAb: monoclonal antibody immunoassay, ROC: receiver operator characteristic

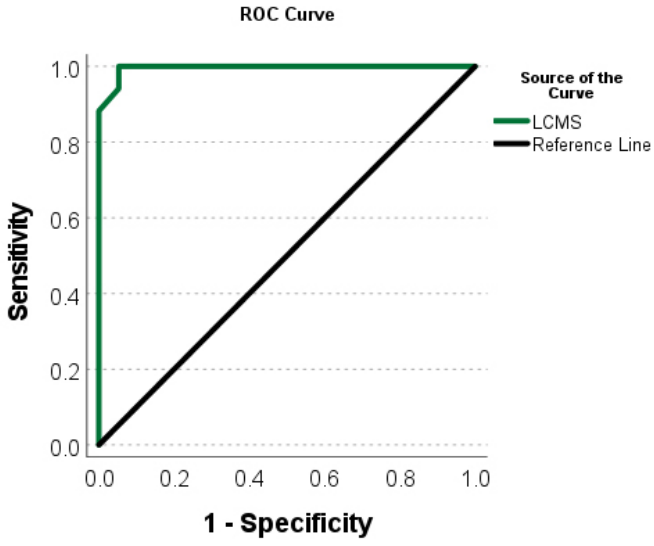

Figure 3. Receiver operating characteristic curve for the diagnosis of adrenal insufficiency based on the peak cortisol level during a 1 mcg Cosyntropin stimulation test measured by LC/MS

LC/MS: liquid chromatography mass spectrometry, ROC: receiver operator characteristic

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 AI 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 to diagnose AI during Cosyntropin stimulation tests in children when newer and highly specific cortisol assays are used. We propose a new peak serum cortisol cutoff value of 12.5 µg/dL and 14 µg/dL when using mAb immunoassay or LC/MS, 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 AI. Our monoclonal and LC/MS assay cutoffs demonstrated a high sensitivity and specificity to ensure that the diagnosis of AI is not missed. We demonstrated a strong kappa correlation coefficient between the traditional peak cortisol level cutoff of 18 µg/dL for the diagnosis of AI 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 valuable information strengthens the rationale to redefine the cutoff for the diagnosis of AI 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 these variables constant adds rigor and reproducibility to the study design and demonstrated that the differences in cortisol levels are assay-specific, leading to cortisol 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).

Study Limitations

One possible limitation of this study was 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 AI, which is also supported by the similar results obtained in our study and results reported from earlier studies (15,16).

Another limitation of this study was 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 AI 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.

Conclusion

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, potentially leading to the overdiagnosis of AI and unnecessary steroid use. Based on our results and those of 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.

Ethics

Ethics Committee Approval: The study was approved by the Washington University in St. Louis of Institutional Review Board (IRB ID: 202012130, date: 08.09.2022).

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

Peer-review: Externally peer-reviewed.

Authorship Contributions

Concept: Samuel Cortez, Ana Maria Arbeláez, Kyle McNerney, Design: Samuel Cortez, Ana Maria Arbeláez, Kyle McNerney, Data Collection or Processing: Samuel Cortez, Ana Maria Arbeláez, Michael Wallendorf, Kyle McNerney, Analysis or Interpretation: Samuel Cortez, Ana Maria Arbeláez, Michael Wallendorf, Kyle McNerney, Literature Search: Samuel Cortez, Ana Maria Arbeláez, Kyle McNerney, Writing: Samuel Cortez, Ana Maria Arbeláez, Michael Wallendorf, Kyle McNerney.

Financial Disclosure: Research reported in this publication was supported by the Washington University Institute of Clinical and Translational Sciences grant UL1TR002345 from the National Center for Advancing Translational Sciences of the National Institutes of Health (NIH) and by the Washington University Pediatric Endocrinology Department. The content is solely the responsibility of the authors and does not necessarily represent the official view of the NIH. S.C., A.M.A., K.M. are part of the Pediatric Endocrinology Department.

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