Clinica Chimica Acta 454 (2016) 112–118

Contents lists available at ScienceDirect

Clinica Chimica Acta

journal homepage: www.elsevier.com/locate/clinchim

Comparison of eight routine unpublished LC–MS/MS methods for the simultaneous measurement of testosterone and androstenedione in serum

Rahel M. Büttler [a], Frans Martens [a], Mariëtte T. Ackermans [b], Andrew S. Davison [c], Antonius E. van Herwaarden [d], Linda Kortz [e], Johannes G. Krabbe [f], Eef G.W. Lentjes [g], Charlotte Syme [h], Rachel Webster [i], Marinus A. Blankenstein [a], Annemieke C. Heijboer [a,*]

[a] Department of Clinical Chemistry, Endocrine Laboratory, VU University Medical Center, Amsterdam, The Netherlands
[b] Department of Clinical Chemistry, Laboratory of Endocrinology, Academic Medical Center, University of Amsterdam, Amsterdam, The Netherlands
[c] Department of Clinical Biochemistry and Metabolic Medicine, Liverpool Clinical Laboratories, Royal Liverpool and Broadgreen University Hospitals Trust, Liverpool, United Kingdom
[d] Department of Laboratory Medicine, Radboud University Medical Centre, Nijmegen, The Netherlands
[e] MVZ wagnerstibbe für Laboratoriumsmedizin, Gynäkologie, Humangenetik und Pathologie GmbH, Göttingen, Germany
[f] Department of Clinical Chemistry and Laboratory Medicine, Medical Spectrum Twente, Medlon BV, The Netherlands
[g] Department of Clinical Chemistry & Haematology, University Medical Center, Utrecht, The Netherlands
[h] Department of Clinical Biochemistry, Glasgow Royal Infirmary, Glasgow, United Kingdom
[i] Department of Clinical Biochemistry, University Hospitals Birmingham NHS Foundation Trust Queen Elizabeth Hospital, Birmingham, United Kingdom

ARTICLE INFO

Article history:
Received 7 December 2015
Received in revised form 2 January 2016
Accepted 4 January 2016
Available online 8 January 2016

Keywords:
LC–MS/MS
Testosterone
Androstenedione
Androgens
Steroid hormones
Method comparison

ABSTRACT

Background: Liquid-chromatography tandem mass spectrometry (LC–MS/MS) has become the method of choice in steroid hormone measurement. However, little information on the mutual agreement of LC–MS/MS methods is available. We compared eight routine unpublished LC–MS/MS methods for the simultaneous measurement of testosterone and androstenedione.
Methods: Sixty random serum samples from male and female volunteers were analysed in duplicate by eight routine LC–MS/MS methods. We performed Passing–Bablok regression analyses and calculated Pearson's correlation coefficients to assess the agreement of the methods investigated with one published method known to be accurate. Intra-assay CV of each method was calculated from duplicate results, recoveries for each method were calculated from six spiked samples. Furthermore, a CV between the investigated methods was calculated.
Results: The concentrations ranged from 0.05–1.26 nmol/L, 6.15–24.44 nmol/L and 0.15–4.78 nmol/L for testosterone in females, testosterone in males and androstenedione, respectively. The intra-assay CVs were between 3.7–16.0%, 0.9–5.2% and 1.2–9.5% for testosterone in females, testosterone in males and androstenedione, respectively. The slopes of the regression lines ranged between 0.90–1.25, 0.87–1.24 and 0.94–1.31 for testosterone concentrations in females, all testosterone values and androstenedione, respectively. Inter-method CVs were 24%, 14% and 29% for testosterone for concentrations in females and males and androstenedione, respectively. These compare unfavourably to the variation found earlier in published methods.
Conclusion: Although most routine LC–MS/MS methods investigated here showed a reasonable agreement, some of the assays showed a high variation. The observed differences in standardization should be taken into account when applying reference values, or should, preferably, be solved.

© 2016 Elsevier B.V. All rights reserved.

1. Introduction

Accurate measurement of hormone concentrations is vital for clinical endocrinology as well as endocrine research. In recent years, more and more attention is paid on the reliability of hormone analysis, especially steroid hormone measurement. Taieb et al. showed that commonly used immunoassays for serum testosterone are not able to reliably measure the low testosterone concentrations in females and children [1]. The editorial, accompanying this publication, stated that these immunoassays are comparable to or even worse than a random number generator in estimating serum testosterone concentrations in females [2]. Since this publication in 2003 [1], an extensive debate on the quality of testosterone assays emerged and resulted in the Endocrine Society's Position Statement and the subsequent Consensus statement on testosterone assays, stating that the accuracy of testosterone measurements needs improvement [3,4].

* Corresponding author at: VU University Medical Center, Department of Clinical Chemistry, De Boelelaan 1117, 1081 HV Amsterdam, The Netherlands.
E-mail address: a.heijboer@vumc.nl (A.C. Heijboer).

http://dx.doi.org/10.1016/j.cca.2016.01.002
0009-8981/© 2016 Elsevier B.V. All rights reserved.

Much effort has since been put in the development of more reliable assays for sex steroids. This resulted in second generation testosterone immunoassays, some of which were found to be more accurate than their predecessors [1,5,6]. Next to this, liquid-chromatography tandem mass spectrometry (LC–MS/MS) is more and more commonly used in steroid hormone analysis to avoid cross reactivity, which is one of the issues in immunoassays [7]. The urgent need for reliable assays as well as the increasing use of LC–MS/MS has prompted the editorial board of the Journal of Clinical Endocrinology and Metabolism (JCEM) to state that from 2015 on '*manuscripts reporting sex steroid assays as important endpoints must use MS-based assays*' and '*it is anticipated that this requirement will be extended to adrenal steroids and vitamin D in the near future*' [8]. Although we fully agree that reliable steroid hormone assays are required and we understand the superiority of LC–MS/MS above immunoassays, we believe that this statement is ambitious, especially because information on the mutual agreement of LC–MS/MS assays is still limited.

Thienpont et al. showed that LC–MS/MS assays for testosterone may agree well with each other and with a reference method and have a low imprecision [9]. However, others showed a less strong agreement and a much higher variation in the investigated LC–MS/MS methods for testosterone [10,11]. Data on the agreement of LC–MS/MS assays for androstenedione are even scarcer [12]. We recently compared seven published LC–MS/MS methods for the simultaneous measurement of testosterone, androstenedione and dehydroepiandrosterone (DHEA) and found that these assays agreed reasonably well. In addition, these published LC–MS/MS assays showed a clearly lower inter-method variation than currently used immunoassays for serum testosterone [13]. These findings support the JCEM statement. However, based upon earlier findings [10,11], it is questionable whether the reported findings apply to unpublished LC–MS/MS methods developed for routine diagnostic use as well. For this reason, we investigated the imprecision, trueness and agreement among eight routine and unpublished LC–MS/MS methods for the simultaneous measurement of testosterone and androstenedione.

2. Materials and methods

2.1. Samples

Sixty random serum samples were obtained from adult volunteers (men and women) presenting at the outpatient clinic of the VU University Medical Center Amsterdam for diagnostic venipuncture in March 2015. There were no further selection criteria and all subjects provided written informed consent. All samples were anonymized and handled identically. After centrifugation the samples were aliquoted and frozen at −20 °C. The samples were sent frozen to the participating laboratories and were kept frozen until analysis. We spiked three female samples with 1 nmol/L testosterone (Riedel de Haën (lot: 5117X), Buchs, Switzerland) and 10 nmol/L androstenedione (Steraloids (lot A6030-100, batch L1712) Newport, RI) and three male samples with 10 nmol/L testosterone and 4 nmol/L androstenedione.

2.2. Method comparison

Eight routine, unpublished methods for the simultaneous measurement of testosterone and androstenedione were included for this comparison study. One published LC–MS/MS was included [12,13]. The methods were randomly coded Method A to Method J, with method A being the published method. All investigated methods were developed and validated in the respective laboratories according to the guidelines adopted by these laboratories. Technical details of the LC–MS/MS methods in this study are shown in Supplemental Data Table 1. The Supplemental Data accompany the online version of this article. In summary, the methods used between 25 and 500 μL serum for singular analysis. Sample preparation consisted of internal standard addition and one or more of the following sample preparation methods: liquid–liquid extraction, protein precipitation using acetonitrile, solid phase extraction and supported liquid extraction. The calibration ranges, Lower Limits of Quantitation (LLOQ) and internal standards used of all methods are shown in Supplemental data Table 2. Some of the methods measure several other steroids besides testosterone and androstenedione. However, in this study only the testosterone and androstenedione results were compared. All samples were analysed in duplicate for testosterone and androstenedione by each of the investigated methods according to the standard procedures concerning calibration and quality control in each of the laboratories. Duplicate measurements were performed in one batch to allow calculation of the intra-assay coefficient of variation (CV) per method. We compared each method to method A, a published method, shown to agree well with six published LC–MS/MS methods for testosterone and androstenedione and to be indirectly comparable with the testosterone reference method [12,13]. All samples were measured in duplicate on two different days using method A. The mean concentration per sample (the mean of the two duplicates) measured by method A was used for further analysis.

2.3. Statistical analysis

We calculated the intra-assay CV per analyte of each method using the following formula: $CV\,\% = \text{square root}\{((\sum (a\text{-}b)^2)/2\,N)(N/\sum\overline{x})\}$, where $\sum$ is the sum, a and b are the duplicate concentrations of the respective method and analyte, N is total number of duplicates and $\overline{x}$ is the mean analyte concentration of a and b. Recoveries were calculated per method using the following formula: $\text{Recovery}\ \% = (([\text{analyte}]_{\text{spiked sample}} - [\text{analyte}]_{\text{sample without addition}}) / [\text{analyte}]_{\text{added}}) * 100\%$. Recoveries are shown as mean ± SD. Using the mean concentrations calculated from the duplicate measurements of each sample, we performed a Passing–Bablok regression analysis and calculated a Pearson's correlation coefficient to assess the agreement of methods B to J with the mean concentration measured by method A. In addition, we calculated the standard deviation (SD) per sample, using the mean concentrations from the duplicate measurements of methods B to J. Inter-method variation was calculated using the following formula: $CV\ (\%) = SD / [\text{analyte}]_{\text{method A}}$. All statistical analyses were performed using MedCalc 11.6, GraphPad Prism 6 and Microsoft Excel 2010.

3. Results

Mean concentrations of testosterone measured by method A were between 0.05–1.26 nmol/L and 6.15–24.44 nmol/L for females (N = 31) and males (N = 29), respectively. There were no samples with a testosterone concentration between 1.26 and 6.15 nmol/L. Mean androstenedione concentrations measured by method A were between 0.15 and 4.78 nmol/L. Comparison of the first and second run of method A revealed the following (data are shown as mean (95% C.I.): slope of the regression line was 1.01 (1.00–1.02), intercept was −0.00 (−0.02 to 0.01) and correlation coefficient 0.999 (0.998 to 0.999) for testosterone and the slope of the regression line was 0.99 (0.95–1.03), intercept was −0.02 (−0.07 to 0.04) and correlation coefficient 0.992 (0.986 to 0.995) for androstenedione. For all eight methods studied, the intra-assay CVs, based on the duplicate measurements, were between 3.7 and 16.0% and 0.9 and 5.2%, for testosterone concentrations in females and in males, respectively. Intra-assay CVs for androstenedione were between 1.2 and 9.5%. Intra-assay CVs per method are shown in Table 1. Recoveries for testosterone were between 96 and 111% for all methods, except methods D and J showing a mean testosterone recovery of only 58% and 64%, respectively. Recoveries for androstenedione were between 85 and 117% for all methods except method J with a mean androstenedione recovery of 63%. Recoveries per method are shown in Table 2. Passing–Bablok regression analyses as well as Pearson's correlation coefficients are shown in Figs. 1–3, for testosterone concentrations in females, all testosterone concentrations and

Download English Version:

https://daneshyari.com/en/article/1965180

Download Persian Version:

https://daneshyari.com/article/1965180

Daneshyari.com