The Androstenedione Roche Elecsys immunoassay has superior comparability to the LC-MS/MS assay than the Siemens Immulite immunoassay

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A B S T R A C T

Androstenedione (ASD) is a biomarker used in the diagnostic workup of hyperandrogenism, congenital adrenal hyperplasia, premature adrenarche, and polycystic ovary syndrome (PCOS). The Elecsys ASD competitive electrochemiluminescence immunoassay (Roche Diagnostics, Indianapolis, IN) is a new assay recently available in the US.

Objective: This study evaluated the analytical and clinical performance of the Elecsys ASD assay.

Design & Methods: We evaluated the linearity/analytical measuring range (AMR), precision, and accuracy of the Elecsys ASD assay on the cobas e601 analyzer. ASD was measured in serum/plasma in the Elecsys ASD, Immulite (Siemens Medical Solutions USA, Inc. Malvern, PA), and LC-MS/MS assays. Reference intervals (RI) were evaluated across genders, menopausal status, and in children. Statistical analysis was performed using EP evaluator and R program.

Results: The Elecsys ASD assay had a linear response across the AMR. The intra- and inter-assay coefficients of variation at various concentrations were ≤4.5%. The Elecsys ASD assay had a mean difference of −0.04 ng/mL (−1.7%) with the LC-MS/MS assay, whereas the Immulite assay had a mean difference of 1.17 ng/mL (66%) and 1.22 ng/mL (38%) compared to the LC-MS/MS and Elecsys ASD assays, respectively. The Roche recommended RIs for healthy men (0.280–1.52 ng/mL) and postmenopausal women (0.187–1.07 ng/mL) were successfully verified. The RIs for children were adopted from published data. For pre-menopausal women, a RI of <1.60 ng/mL was established. The ASD concentrations in women with and without PCOS overlapped.

Conclusions: The Elecsys ASD assay has superior comparability to the LC-MS/MS assay than the Immulite assay.

1. Introduction

Androstenedione (ASD) is the precursor of testosterone and estrone and is produced in the gonads and adrenal glands. It is derived from 17-hydroxypregosterone by 17α-hydroxylase/17, 20-lyase, and dehydroepiandrosterone (DHEA) by 3β-hydroxysteroid dehydrogenase. Along with other sex hormones, ASD is essential for the onset and development of sexual differentiation in males and females. ASD measurement in serum or plasma is used to assess adrenal and gonadal function and steroid hormone biosynthesis...
deficiency. It is a valuable biomarker for investigating virilizing endocrinopathies and in the diagnostic workup of a subset of patients with polycystic ovary syndrome (PCOS). ASD measurement also complements the evaluation of steroidogenesis disorders, especially in diagnosing and monitoring pediatric patients with hyperandrogenic syndromes such as classical and non-classical congenital adrenal hyperplasia and premature adrenarche [1–3]. Recently, an emerging role of ASD in adrenal vein sampling has also been proposed [4].

The gold standard for ASD measurement is liquid chromatography-tandem mass spectrometry (LC-MS/MS). Until recently, the Immulite ASD assay (Siemens Medical Solutions USA, Inc. Malvern, PA) was the only non-radioimmunologic immunoassay available in the US [5]. The DiaSorin LIAISON ASD chemiluminescent immunoassay shows good comparability with the gold standard, however, it is not available to use in the US [6]. According to a recent College of American Pathologists survey, approximately 50% of the laboratories that test for ASD use the Immulite assay. However, while this immunoassay is automated and relatively easy to perform, it overestimates the concentration of ASD compared to LC-MS/MS [6].

In 2020, the Food and Drug Administration cleared the Roche Diagnostics’ (Indianapolis, IN) Elecsys ASD assay [7]. The assay is a competitive electrochemiluminescence immunoassay that uses monoclonal antibodies for ASD detection. This study aimed to evaluate the performance characteristics of the Elecsys ASD assay and to compare it to the widely used Immulite ASD assay and an LC-MS/MS assay.

2. Material and methods

2.1. Reagents, patient samples, and assays

The Elecsys ASD assay was performed using the cobas e601, and the Immulite ASD was performed using the Immulite 2000. Specimens were sent to a reference laboratory for analysis by LC-MS/MS [8]. ASD standard was purchased from Sigma-Aldrich (St. Louis, MO). Residual de-identified serum and lithium heparin and EDTA plasma specimens were utilized in the study. Regents and specimens were handled in accordance with the method sheets. Calibration verification materials (CalCheck ASD) and quality control (QC) (PreciControl Maternal Care Control) were obtained from Roche Diagnostics. This study was approved by the Institutional Review Board.

The Siemens Immulite 2000/Xpi assay is a 63-min competitive chemiluminescent immunoassay that employs anti-ASD polyclonal antibodies and alkaline phosphatase-conjugated ASD [5]. The Elecsys ASD assay is an 18-min competitive electrochemiluminescence immunoassay that utilizes ASD-specific biotinylated monoclonal antibodies and ruthenium complex-conjugated ASD for ASD assessment. Although this is a biotin-based assay, it has been optimized to minimize biotin interference. Biotin concentrations up to 3500 mg/dL or 14326 nmol/L were tested by Roche, and no interference was found [9].

2.2. Analytical measuring range (AMR)

The linearity of the assay across the AMR of 0.3–10 ng/mL (1.0–34.9 nmol/L) was evaluated using the Roche cobas CalCheck ASD set in triplicate. The analytical measurement range (AMR) was performed with matrix-appropriate materials according to recommendations by the College of American Pathology (CAP) checklist and CLSI guidelines [10,11]. The set, a commercial assayed control of 5 lyophilized ASD concentrations in human serum, is recommended by the method manufacturer as a suitable material [12]. The limit of quantitation was used to set the lower limit of the AMR. The limit of detection of the assay is 0.150 ng/mL (0.525 nmol/L).

2.3. Precision

The intra-assay and inter-assay precision were evaluated using 3 patient pools and QC material at 3 different concentrations. Intra-assay precision consisted of analyzing 10 consecutive replicates of patient pools and QC. Inter-assay precision was evaluated 2 times per day for 10 days for the QC and 3 times per day for 5 days for the patient pools to minimize stability variables [13].

2.4. Accuracy by method comparison

We used 40 specimens (33 serum and 7 plasma) for the method comparison studies, the minimum recommended by CLSI EP09c [14]. The specimens were residual de-identified samples originally tested for ASD (n = 34) and a de-identified serum pool spiked with ASD Certified Reference Material (Cerilliant, TX) at concentrations covering the AMR (n = 6). ASD was measured in serum and plasma specimens using the Elecsys ASD and the LC-MS/MS assays. The CV% of the LC-MS/MS was ≤11.2 and it showed agreement with other LC-MS/MS assays [8]. Only serum specimens were tested by the Immulite ASD assay because plasma is not an acceptable specimen type. A serum pool was spiked with an ASD standard to achieve higher concentrations and span the AMR. Deming regression, absolute difference, and percent difference Bland Altman plots were created using the "mcr" and "ggExtra" packages in the R program (RStudio Team, 2020).

2.5. Reference interval (RI)

To verify the expected values provided by Roche for healthy men (0.280–1.52 ng/mL or 0.979–5.32 nmol/L, central 95%), healthy women (0.490–1.31 ng/mL or 1.71–4.58 nmol/L, central 90%), postmenopausal women (0.187–1.07 ng/mL or 0.654–3.74 nmol/L,
central 95%), and healthy children (<0.519 ng/mL or <1.81 nmol/L, central 95%) [9], at least 20 specimens from apparently healthy individuals per group were tested as per CLSI guidelines [15]. We used residual specimens from patients who presented for routine health checkups and excluded patients with chronic conditions and endocrinopathies (e.g., abnormal thyroid and adrenal disease), significant weight changes, and history of tobacco use. In pre-menopausal women, the use of hormone treatments such as Levonorgestrel Ethinyl Estradiol, Depo-Provera, NEXPLANON, etc., was documented. The patients were carefully screened by chart review. Women 47-years-old and older were included in the postmenopausal group. Roche also lists a RI for patients with PCOS (0.645–3.47 ng/mL or 2.26–12.1 nmol/L, central 95%), which we evaluated in samples from female patients diagnosed with PCOS (as indicated in their medical record). RIs were successfully verified for each group if less than 10% of samples failed. If 3–4 failed the reported limits, an additional 20 clinical defined specimens were run. If >4 failed, we established the RI using specimens from at least 120 individuals, when possible.

2.6. Statistical analysis

Data were analyzed using EP evaluator (Data Innovations, Colchester, VT) or the R program (RStudio Team, 2020). A total allowable error (TAE) of 22% or 0.57 ng/mL (2.0 nmol/L) was used based on the biological variation of ASD [16]. Fifty percent of the TAE (11%, 0.29 ng/mL or 1.0 nmol/L, whichever is greater) was budgeted for bias and 25% of the TAE (5.5%, 0.14 ng/mL or 0.5 nmol/L, whichever is greater) was budgeted for precision.

3. Results

3.1. AMR

The Roche assay had a linear response across the AMR (0.3–10.0 ng/mL or 1.0–34.9 nmol/L) with a correlation coefficient of 0.951 and a y-intercept of 0.078. The coefficients of variation (CVs) were 0.5%–1.8%, and analytical recoveries were 94.5%–109.9% across the tested concentrations.

3.2. Precision

The assay had excellent precision at all tested concentrations. The inter-assay and intra-assay CVs were all within 3.9%. The intra-assay CVs for QC and the patient pools were 1.5%–2.0% and 1.2%–1.9%, respectively. The inter-assay CVs for QC and the patient pools were 2.5%–3.5% and 1.8%–3.9%, respectively. The total CVs were within 4.5% for all samples tested. A detailed list of the data is provided in Table 1.

3.3. Method comparison

Deming regression of the LC-MS/MS vs. the Elecsys ASD assays was Elecsys ASD = 1.00 * LC-MS/MS - 0.04, r = 0.995 (n = 40) (Fig. 1A). The absolute and percent difference between the two methods were −0.04 ng/mL and −1.7%, respectively, as shown in the corresponding Bland-Altman plots (Fig. 1B and C). These analyses indicated that the two assays had an excellent correlation. Conversely, the comparison of the Immulite ASD assay with the Elecsys ASD and the LC-MS/MS assays showed the Siemens’ assay exhibits good correlation (>0.95) but not comparability because of bias (Fig. 1D and G). Deming regression of the Immulite and the Elecsys ASD assays was Elecsys ASD = 0.79 * Immulite - 0.43, r = 0.975 (n = 33; serum only) (Fig. 1D) and the Deming regression of the LC-MS/MS and the Immulite Assays was Immulite = 1.27 * LC-MS/MS + 0.46, r = 0.973 (n = 33; serum only) (Fig. 1G). Results by the Immulite were different by 1.22 ng/mL (−38%) and 1.17 ng/mL (66%) in average compared to Roche Elecsys ASD assay and LC-MS/MS, respectively (Fig. 1H and I).

Table 1

| Material | QC (n = 10) | Patient Pool (n = 10) |
|----------|------------|----------------------|
| Level    | Low        | Medium | High | Low | Medium | High |
| Mean (ng/mL) | 0.601     | 2.95 | 6.61 | 0.475 | 1.25 | 4.11 |
| Within Run %CV | 2.0       | 1.5  | 1.6  | 1.9  | 1.2  | 1.5  |
| Inter-Assay Precision | Patient Pool (n = 15) |
| Level    | Low        | Medium | High | Low | Medium | High |
| Mean (ng/mL) | 0.602     | 2.95 | 6.66 | 0.499 | 1.31 | 4.11 |
| Between Run %CV | 3.5       | 3.3  | 2.5  | 3.9  | 2.7  | 1.8  |
| Total %CV | 3.7        | 3.3  | 2.5  | 4.5  | 3.1  | 2.4  |
Fig. 1. Deming regression, Bland-Altman difference and percent difference plots of three androstenedione assays. Roche Elecsys ASD vs. LC-MS/MS Deming regression (A), Bland-Altman difference and percent difference plots (B–C); Roche Elecsys ASD vs. Siemens Immulite ASD Deming regression (D), Bland-Altman difference and percent difference plots (E–F); Siemens Immulite ASD vs. LC-MS/MS Deming regression (G), Bland-Altman difference and percent difference plots (H–I). In the Bland-Altman plots, the solid thin lines represent the mean, the dotted lines represent 2 SD of the mean, the horizontal histograms show the distribution of the average ASD concentrations, and the vertical histograms show the distribution of the ASD difference and percent difference by the two assays.

Table 2
Reference interval (RI) verification status and alternative solutions for each gender and age group.

| Test Subjects, Roche Method Sheet | Expected values, Roche Method Sheet (ng/mL) | Verified? | Follow up |
|-----------------------------------|--------------------------------------------|-----------|-----------|
| Apparently healthy men (n = 138, central 95%) | 0.280–1.520 | Yes | Not applicable |
| Postmenopausal women (n = 140, central 95%) | 0.187–1.070 | Yes | Not applicable |
| Apparently healthy children (n = 140, central 95%) | <0.519 | No | Adopted the RIs established using a comparable assay [8] |
| Apparently healthy women (n = 84, central 90%) | 0.490–1.310 | No | Established a new RI using 120 samples from apparently healthy individuals |
| Women with PCOS (n = 125, central 95%) | 0.645–3.47 | No | Evaluated 38 samples from patients with PCOS. A separate RI was not established since it may not be clinically necessary |
3.4. Reference interval (RI)

The expected values listed by Roche in the assay method sheets for healthy men (0.280–1.52 ng/mL or 0.979–5.32 nmol/L, central 95%) and postmenopausal women (0.187–1.07 ng/mL or 0.654–3.74 nmol/L, central 95%) were successfully verified (Table 2). However, expected values verification for other groups was not successful.

For premenopausal women, a RI of <1.6 ng/mL (<5.6 nmol/L) was established using 120 specimens from apparently healthy women (Fig. 2). Finally, 38 de-identified residual specimens from women diagnosed with PCOS were tested and compared to the expected values listed by Roche; verification of the expected values was not successful. While the observed concentrations were significantly different from concentrations observed in apparently healthy women from a statistical perspective (p = 0.047), they were indistinguishable from a clinical perspective due to substantial concentration overlap (Fig. 2).

The verification of the pediatric group reference interval failed. Among 21 specimens (age range: 22 months to 17 years of age), 8 specimens (ages 12–16 years of age) had ASD concentrations ranging from 0.526 to 1.140 ng/mL, which was greater than the recommended RI (<0.519 ng/mL or <1.81 nmol/L, central 95%). This warranted establishing a new RI for this group, which is very challenging for most institutions. Moreover, ASD concentrations change across age groups in childhood and by gender [8]. Instead, we adopted the pediatric RIs established by the reference lab using the LC-MS/MS assay [8,15]. The Roche Elecsys assay and the LC-MS/MS assay from the reference lab showed excellent comparability, and the population of a reference lab is usually diverse and likely reflects our population [8].

4. Discussion

The Elecsys assay has an AMR of 0.3–10.0 ng/mL (1.0–34.9 nmol/L) and demonstrated outstanding precision with patient specimens and quality control materials. It surpassed the Immulite assay performance with excellent comparability to an LC-MS/MS assay. Similar to other studies [6,17], our data demonstrated that the Immulite assay correlated to an LC-MS/MS assay, but it overestimated ASD concentration. Such over-recovery of the assay could be due to differences in calibration. However, further investigation is needed. According to the vendor’s method sheet, the Elecsys assay utilizes monoclonal anti-ASD antibodies and shows significantly less cross-reactivity to other naturally occurring steroids or therapeutic drugs that may be present in patient samples [9]. Moreover, the assay requires significantly lower testing volume, which may benefit certain populations, such as pediatrics.

ASD measurement by LC-MS/MS remains the gold standard, although the Elecsys assay offers a robust analytical alternative for laboratories that need to use an immunoassay. The typical advantages of the latter approach include cost savings and reduced turnaround time relative to sending the test out, and the ease of testing associated with an automated immunoassay. However, the Elecsys ASD assay does have a few limitations. Similar to many other immunoassays, the Elecsys ASD assay may be prone to interference from heterophilic antibodies, anti-streptavidin antibodies, ruthenium or the idiotype of the ruthenium labeled antibody [18]. The limit quantification of the Elecsys ASD assay is 0.3 ng/mL (1.0 nmol/L), which may not be sensitive enough to detect minor elevations in pediatric patients younger than 7 years of age. Although the lower limit of detection of the assay is 0.15 ng/mL (0.5 nmol/L), the quantification may not be as reproducible [9]. This may be clinically insignificant but it remains to be investigated further. ASD is used as a biochemical marker for adequate treatment in pediatric patients with CAH although guidance about specific targets is not provided [19]. Adequately treated patients may have normal or mildly elevated values [19–21], and there is a general consensus to target ASD concentrations within age-adjusted reference intervals [20,21].

RIs for apparently healthy men and postmenopausal women were successfully verified. However, verification of the RIs for

![Fig. 2. Androstenedione concentration in the specimens from the healthy women and the PCOS groups.](image-url)
pediatric patients, apparently healthy women, and women with PCOS were unsuccessful (Table 2). As a solution, RIs were either adopted, re-established, or not used (i.e., when there was no clinical value). The manufacturer-suggested expected values were established for the pediatric group using samples from 140 children ages 2–8 years of European descent (personal communication). This population does not represent the pediatric population we serve, potentially explaining the discrepancy in observed results. In addition, ASD changes over time in this age group, and a single RI does not adequately represent the changes. In our verification study, the pediatric specimens outside of the vendor’s proposed reference interval were from older pediatric patients (12-16 year-old). The age of the patients in our study also likely contributed to differences observed in our verification samples. Ideally, a new RI for this group should be established using 120 healthy patient specimens. However, the lack of qualified samples and the fact that ASD concentration changes throughout different pediatric age groups make this option challenging and impractical. Alternatively, since the pediatric patient demographics and the methods were comparable, RIs by sex, age, and Tanner stage established using the LC-MS/MS assay were adopted [8].

The manufacturer’s reported expected values for premenopausal women were based on a sample size of 84 individuals and the central 90th percentile instead of the central 95th percentile. Not surprisingly, we could not verify the RI. As a solution, we established a new RI in apparently healthy women. In addition, whether the patient was taking hormone treatments such as contraceptive medication at the time of the blood draw was noted. Interestingly, contrary to the data from another study, which suggested oral contraceptives significantly lowered serum concentrations of all steroid metabolites including ASD [22], ASD concentrations from these two subgroups were not significantly different in our cohort (p = 0.075) (Fig. 2). Therefore, a single RI of <1.6 ng/mL (<5.6 nmol/L) was established for this group. RI verification in women with PCOS also failed. However, compared to the concentrations in apparently healthy women, while ASD concentrations from this cohort were significantly different (p = 0.047), they were indistinguishable from a clinical perspective due to substantial concentration overlap (Fig. 2). As a result, we decided not to establish a separate RI for this group since it is not clinically significant. Manufacturers’ method sheets routinely recommend that laboratories investigate the transferability of the expected values in their patient population and determine their own reference intervals if necessary. Our findings highlight the importance of laboratories scrutinizing the patient population and statistical analyses used in the studies by manufacturers and the need to verify reference intervals during the validation process.

One of our study’s limitations was the use of residual specimens from apparently healthy patients for the reference interval studies. Although the screening process was stringent, it may not be the optimal solution for gathering specimens for populations such as the pediatric group. Unlike healthy adults, healthy children are less likely to undergo blood collection. Because of this limitation, we were unable to find enough qualified specimens in a timely manner for this age group. Unfortunately, since the Roche Elecsys assay is relatively new, national RI initiatives such as the Canadian Laboratory Initiative on Pediatric Reference Intervals (CALIPER) had not included the relevant data when this study was performed [23]. In addition, specimen recruitment for the women with PCOS group was also based on chart reviews. However, PCOS is a complex disorder that is highly heterogeneous. The included patients vary in diagnostic history, diagnostic method, age, and treatments. Since some of the women were being treated, we were able to observe the concentration that would be observed at diagnosis in presumably un-treated women. Furthermore, the ASD levels of these patients could be associated with many factors such as obesity and dysglycemia [24,25]. Therefore, a separate RI may not provide any clinically meaningful information. The clinical utility of this new assay in the diagnostic workup of PCOS awaits further investigation.

In summary, the Elecsys ASD assay has excellent precision, specificity, and accuracy. It offers many advantages over the Immulite ASD assay and is a suitable alternative for ASD measurement in clinical laboratories. However, the assay should be thoroughly validated before its use for clinical testing and reference intervals established for the patient population, as needed.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

[1] R. Jeelani, M. Bluth, Reproductive function and pregnancy, in: S. Melmed, R. Koenig, C. Rosen, R. Auchus, A. Goldfine (Eds.), Williams Textbook of Endocrinology, fourteenth ed., Elsevier, 2019.
[2] M.G. Ballerini, V. Gaido, M.E. Rodriguez, A. Chiesa, M.G. Ropelato, Prospective and descriptive study on serum androstenedione concentration in healthy children from birth until 18 Years of age and its associated factors, Dis. Markers (2017), https://doi.org/10.1155/2017/9238304.
[3] R. Nerenz, E. Junghim, A. Gronowski, Reproductive endocrinology and related disorders, in: N. Rifai (Ed.), Tietz Textbook of Clinical Chemistry and Molecular Diagnostics, sixth ed., Elsevier, 2018.
[4] G. Ceolotto, G. Antonelli, G. Maiolino, et al., Androstenedione and 17-α-Hydroxyprogesterone are better indicators of adrenal vein sampling selectivity than cortisol, Hypertension 70 (2) (2017) 342–346, https://doi.org/10.1161/HYPERTENSIONAHA.117.09415.
[5] S.H. Diagnostics, Siemens Immulite 2000 Androstenedione, 2018.
[6] J. van Helden, R. Weiskirchen, Cross-method comparison of serum androstenedione measurement with respect to the validation of a new fully automated chemiluminescence immunoassay, Clin. Biochem. 62 (2018) 32–38, https://doi.org/10.1016/j.clinbiochem.2018.09.009.
[7] Elecsys Androstenedione 07679840190. FDA Report.
[8] M.M. Kushnir, T. Blamires, A.L. Rockwood, et al., Liquid chromatography-tandem mass spectrometry assay for androstenedione, dehydroepiandrosterone, and testosterone with pediatric and adult reference intervals, Clin. Chem. 56 (7) (2010) 1138–1147, https://doi.org/10.1373/clinchem.2010.143222.
[9] Roche Diagnostics, Elecsys androstenedione. Version 2.0. https://pm-eservices.roche.com/ltd/api/downloads/34287664-0951-4c11-09f1-005056a71a5d?countryIsoCode=us.
[10] R. McEnroe, A. Durham, M. Kondratovich, et al., EP06 A Evaluation of Linearity of Quantitative, second ed., Clinical and Laboratory Standards Institute, 2020.
[11] Chemistry and Toxicology Checklist, CAP Accreditation Program, Northfield, IL, 2021, 2021.
[12] Cobas, CalCheck Androstenedione, Indianapolis, 2020.
[13] R. McEnroe, A.P. Durham, M.D. Goldford, et al., CLSI EP05-A3 : 2014 Evaluation of Precision of Quantitative Measurement Procedures, third ed., Approved Guideline —, 2014.

[14] J. Budd, A. Durham, T. Gwise, et al., EP09c: Measurement Procedure Comparison and Bias Estimation Using Patient Samples, 2018.

[15] G. Horowitz, S. Altaie, J. Boyd, et al., EP28-A3c: Defining, Establishing, and Verifying Reference Intervals in the Clinical Laboratory; Approved Guideline, third ed., CLSI Document EP28-A3c., 2010.

[16] C. Fraser, Biological Variation: from Principles to Practice, first ed., AACC Press, 2001.

[17] B. Obermayer-Pietsch, R. Im Dahl, M. de Ramon, et al., Multicentre performance evaluation of the new, fully automated Elecsys ASD immunoassay and determination of reference ranges, in: Endocrine Abstracts, 2020, https://doi.org/10.1530/endoabs.70.AEP837.

[18] L.K. Dahll, E.M. Haave, S.R. Dahl, F.E. Aas, P.M. Thorby, Endogenous anti-streptavidin antibodies causing erroneous laboratory results more common than anticipated, Scand. J. Clin. Lab. Invest. 81 (2) (2021) 92–103, https://doi.org/10.1080/00365513.2020.1858493.

[19] P.W. Speiser, W. Arlt, R.J. Auchus, et al., Congenital adrenal hyperplasia due to steroid 21-hydroxylase deficiency: an endocrine society clinical practice guideline, J. Clin. Endocrinol. Metab. 103 (11) (2018) 4043–4088, https://doi.org/10.1210/JC.2018-01865.

[20] C.S. Clausen, M.L. Ljubicic, K.M. Main, et al., Congenital adrenal hyperplasia in children: a pilot study of steroid hormones expressed as sex- and age-related standard deviation scores, Horm. Res. Paediatr. 93 (4) (2020) 226–238, https://doi.org/10.1159/000509079.

[21] A. Nordenstrom, H. Falhammar, Management of endocrine disease: diagnosis and management of the patient with non-classic CAH due to 21-hydroxylase deficiency, Eur. J. Endocrinol. 180 (3) (2019) R127–R145, https://doi.org/10.1530/EJE-18-0712.

[22] T. Søeborg, H. Frederiksen, A. Mouritsen, et al., Sex, age, pubertal development and use of oral contraceptives in relation to serum concentrations of DHEA, DHEAS, 17α-hydroxyprogesterone, 5α-androstenedione, testosterone and their ratios in children, adolescents and young adults, Clin. Chim. Acta 437 (2014) 6–13, https://doi.org/10.1016/j.cca.2014.06.018.

[23] CALIPER pediatric reference interval database. https://caliper.research.sickkids.ca/#/.

[24] G. Misichronis, N.A. Georgopoulos, D.J. Marioli, et al., The influence of obesity on Androstenedione to Testosterone ratio in women with polycystic ovary syndrome (PCOS) and hyperandrogenemia, Gynecol. Endocrinol. 28 (4) (2012) 249–252, https://doi.org/10.3109/09513590.2011.613965.

[25] M.W. O’Reilly, A.E. Taylor, N.J. Crabtree, et al., Hyperandrogenemia predicts metabolic phenotype in polycystic ovary syndrome: the utility of serum androstenedione, J. Clin. Endocrinol. Metab. 99 (3) (2014) 1027–1036, https://doi.org/10.1210/jc.2013-3395.