P1-P138

MEASUREMENTS OF ESTRADIOL AND TESTOSTERONE IN UMBILICAL CORD BLOOD BY GAS CHROMATOGRAPHY-TANDEM MASS SPECTROMETRY (GC-MS/MS); COMPARISONS WITH RADIOIMMUNOASSAY (RIA)

Contact information: kerstin.allvin@vgregion.se

Kerstin Allvin[1,2], Jovanna Dahlgren[1], Mats X Andersson[1,3], Carina Ankarberg-Lindgren[1]

[1]Gothenburg Pediatric Growth Research Center, Department of Pediatrics, Institute of Clinical Sciences, Sahlgrenska Academy, University of Gothenburg, Gothenburg, Sweden

[2]Division of Neonatology, Department of Pediatrics, Institute of Clinical Sciences, Sahlgrenska Academy, University of Gothenburg, Gothenburg, Sweden

[3]Department of Biological and Environmental Sciences, University of Gothenburg, Gothenburg, Sweden

Background

We have previously shown radioimmunoassay (RIA) and gas chromatography-tandem mass spectrometry (GC-MS/MS) to be comparable when analyzing estradiol and testosterone concentrations in prepubertal and pubertal children. However, the reliability for steroid hormone determination with RIA in umbilical cord blood is not known. In general, older studies using RIA show higher values of testosterone, than more recent ones using mass spectrometry.

Patients and methods

Umbilical cord blood was collected from 236 infants (133 boys, 103 girls). Estradiol and testosterone concentrations were analyzed using both RIA (Spectria, Orion Diagnostica, Espoo, Finland) and GC-MS/MS (Agilent, Montreal, Canada).

For estradiol the limit of detection (LOD) was 9 pmol/L for RIA and 2 pmol/L for GC-MS/MS. With RIA the intra-assay coefficient of variation (CV) was <8% whereas the interassay CV was <13% for concentrations ≥ 40 pmol/L. With GC-MS/MS the intra-assay CV was <4% and the interassay CV was 7% for concentrations ≥300 pmol/L.

For testosterone the LOD was 0.1 nmol/L for both RIA and GC-MS/MS. With RIA the intra- and interassay CV was < 7% for concentrations of ≥ 0.9 nmol/L. With GC-MS/MS the intra-assay CV was <4% and the interassay CV was 16% for 0.2 nmol/L and ≤8% for concentrations of ≥5 pmol/L. Correlation analyses were made with Pearson correlation.

Results

For estradiol, there was a good correlation between RIA and GC-MS/MS (r=0.92, p=0.000) (Figure 1). The concentrations of estradiol were similar when measured with RIA versus GC-MS/MS (Table 1). For testosterone, there was also a correlation between RIA and GC-MS/MS (r=0.44, p=0.000) (Figure 3). Umbilical cord blood testosterone concentrations determined with RIA were higher than with GC-MS/MS (Table 1), with linear fit functions; RIA = 0.99 x GC-MS/MS + 3.10 nmol/L.

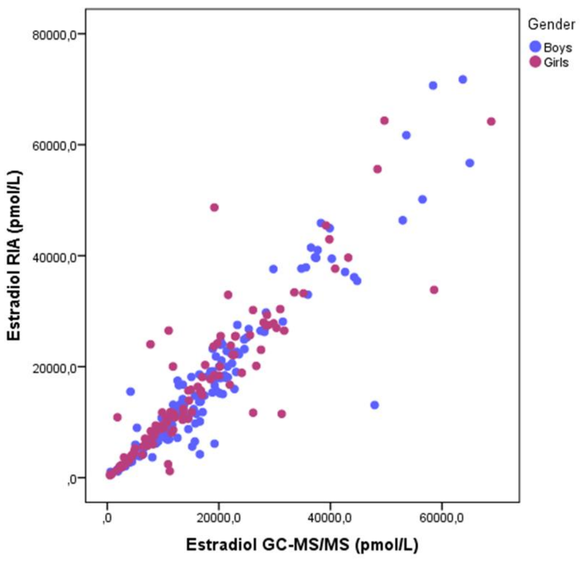

Figure 1. Estradiol RIA vs. estradiol GC-MS/MS. The correlation between estradiol RIA and estradiol GC-MS/MS was high in boys (r=0.94, p=0.000) and in girls (r=0.89, p=0.000).

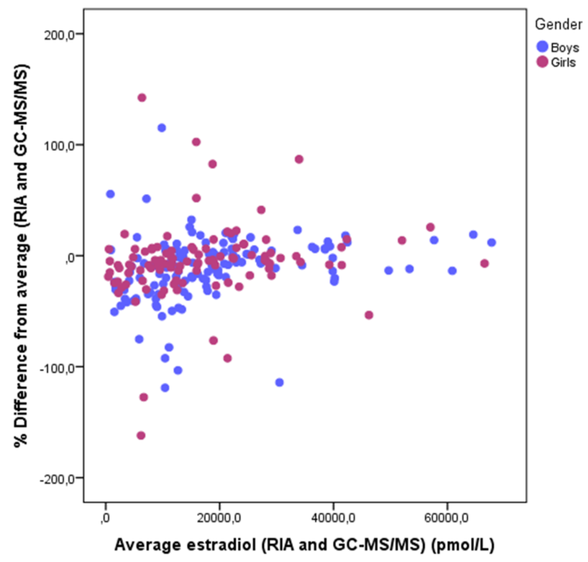

Figure 2. Bland-Altman plot for Estradiol RIA and Estradiol GC-MS/MS.

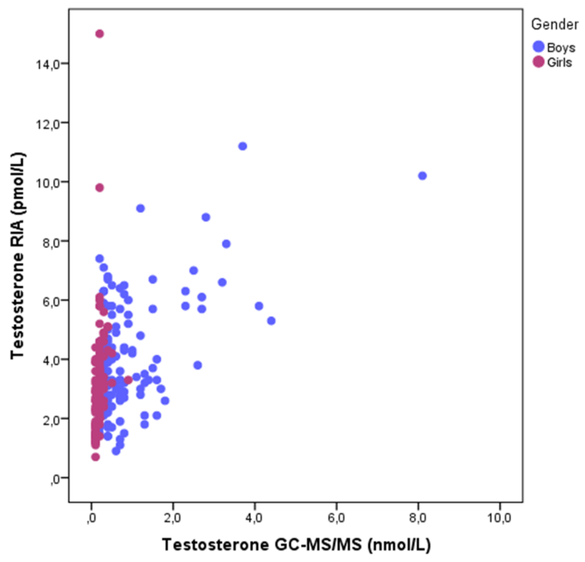

Figure 3. Testosterone RIA vs. testosterone GC-MS/MS. The correlation was higher for boys (r=0.51, p=0.000), than girls (r=0.29, p=0.003).

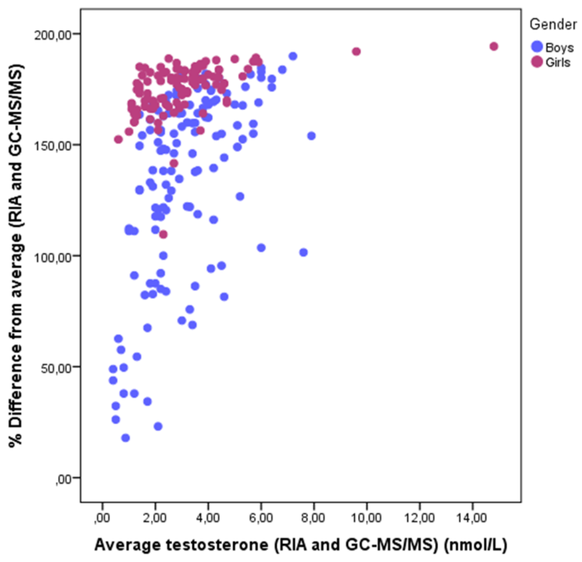

Figure 4. Bland-Altman plot for Testosterone RIA and Testosterone GC-MS/MS.

Table 1. Data is expressed as medians with range in parentheses.

	Boys (n=133)	Girls (n=103 for estradiol, n=101 for testosterone)
Estradiol RIA (pmol/L)	14770 (939 – 71765)	12442 (413 – 64348)
Estradiol GC-MS/MS (pmol/L)	16600 (588 – 64987)	14457 (499 – 68825)
Testosterone RIA (nmol/L)	3.6 (0.9 – 11.2)	3.1 (0.7 – 15.0)
Testosterone GC-MS/MS (nmol/L)	0.5 (0.2 – 8.1)	0.2 (0.1 – 0.9)

Conclusion

We conclude that RIA is sufficient for determination of estradiol, but not testosterone, in umbilical cord blood.

Discussion

Although RIA is as reliable as GC-MS/MS when analyzing testosterone in serum from prepubertal and pubertal children, that is not the case with umbilical cord blood. We speculate that this is due to cross reactivity with other androgens, testosterone metabolites, or estrogens when analyzing testosterone with RIA.

References:

1. Hollier LP, Keelan JA, Hickey M, Maybery MT, Whitehouse AJ.
Front Endocrinol (Lausanne). 2014 May 2;5:64. Measurements of androgen and estrogen concentrations in cord blood: accuracy, biological interpretation, and applications to understanding human behavioral development.

2. **Ankarberg-Lindgren** C, Dahlgren J, Andersson MX.
J Steroid Biochem Mol Biol. 2018 Oct;183:116-124. High-sensitivity quantification of serum androstenedione, testosterone, dihydrotestosterone, estrone and estradiol by gas chromatography-tandem mass spectrometry with sex- and puberty-specific intervals.