

Introduction

Cases of 46,XY DSD present a greater complexity for an etiological definition. The measurement of steroid level is one of the challenges in pediatric endocrinology. Liquid chromatography associated with tandem mass spectrometry (LC-MS/MS) is currently considered the gold standard for steroid measurement, although immunoassay (IA) methods have been used for years.

Objective

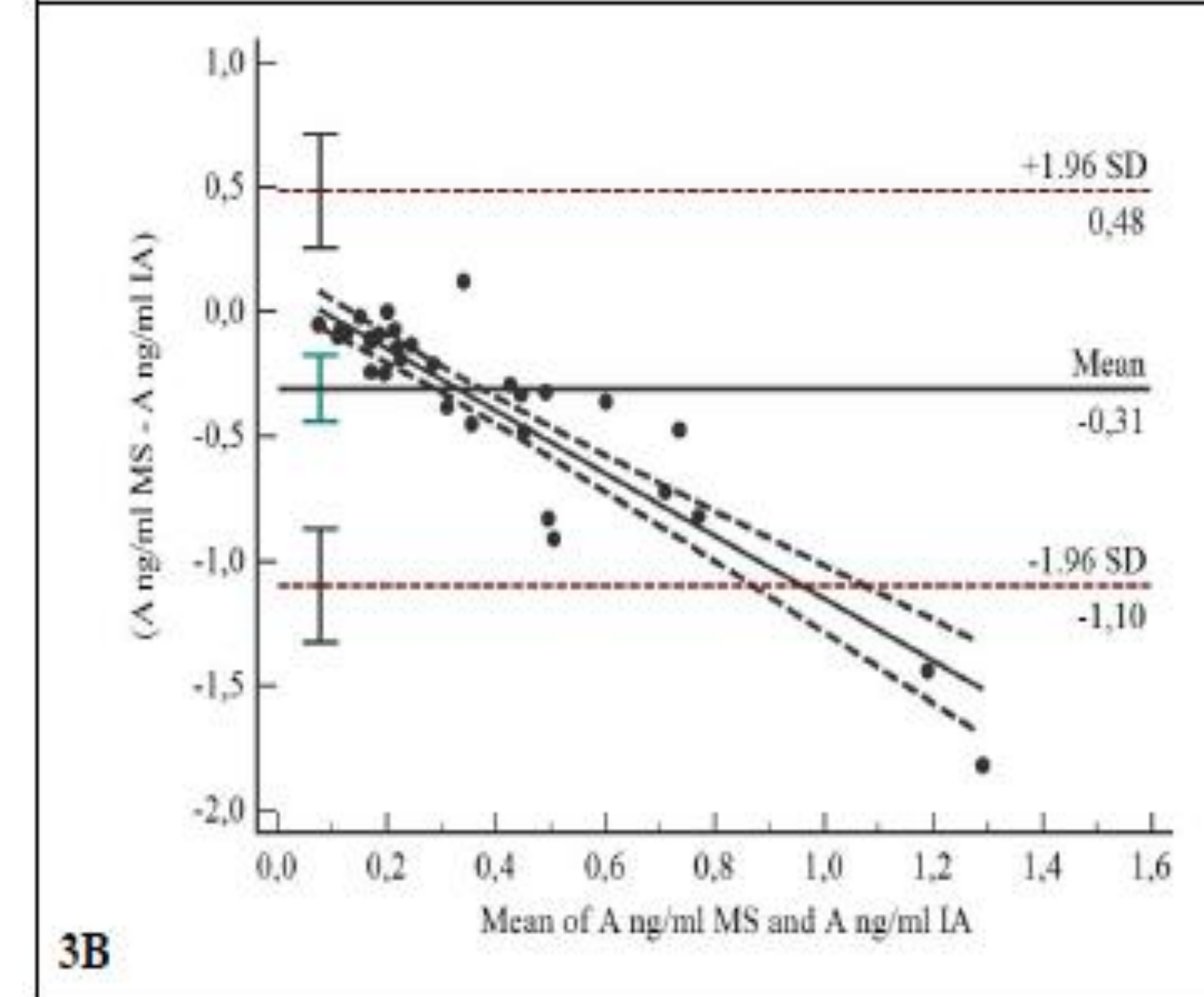
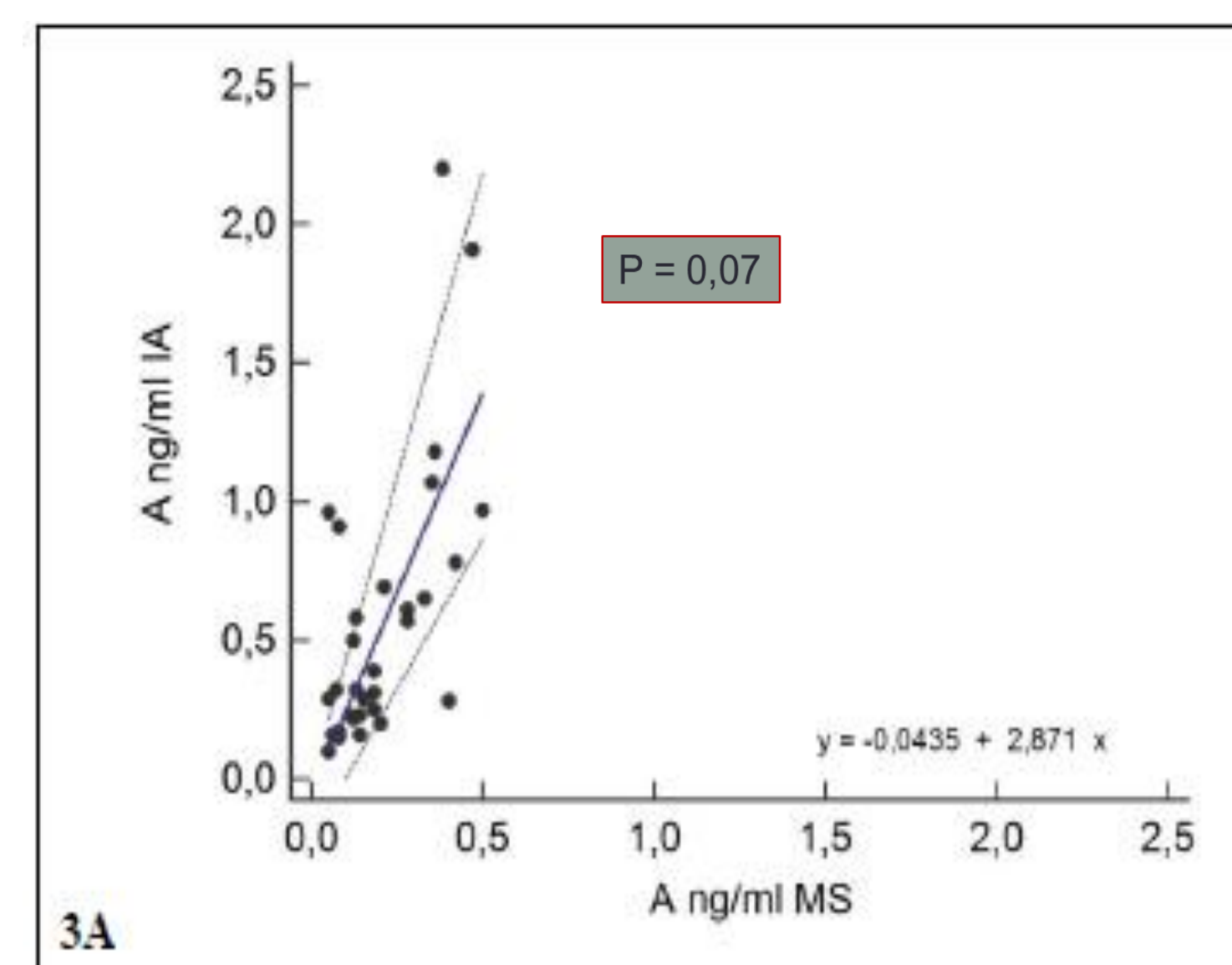
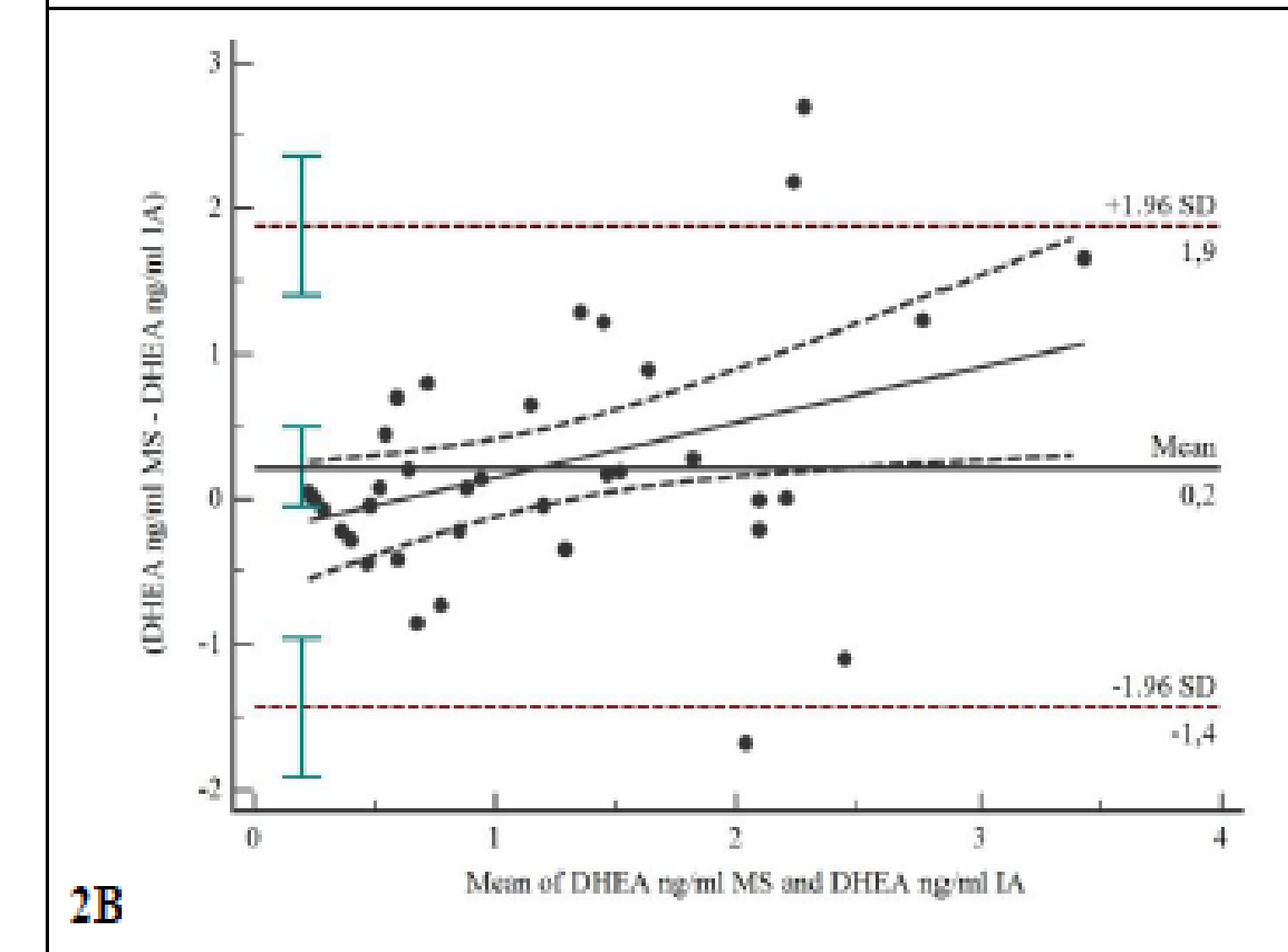
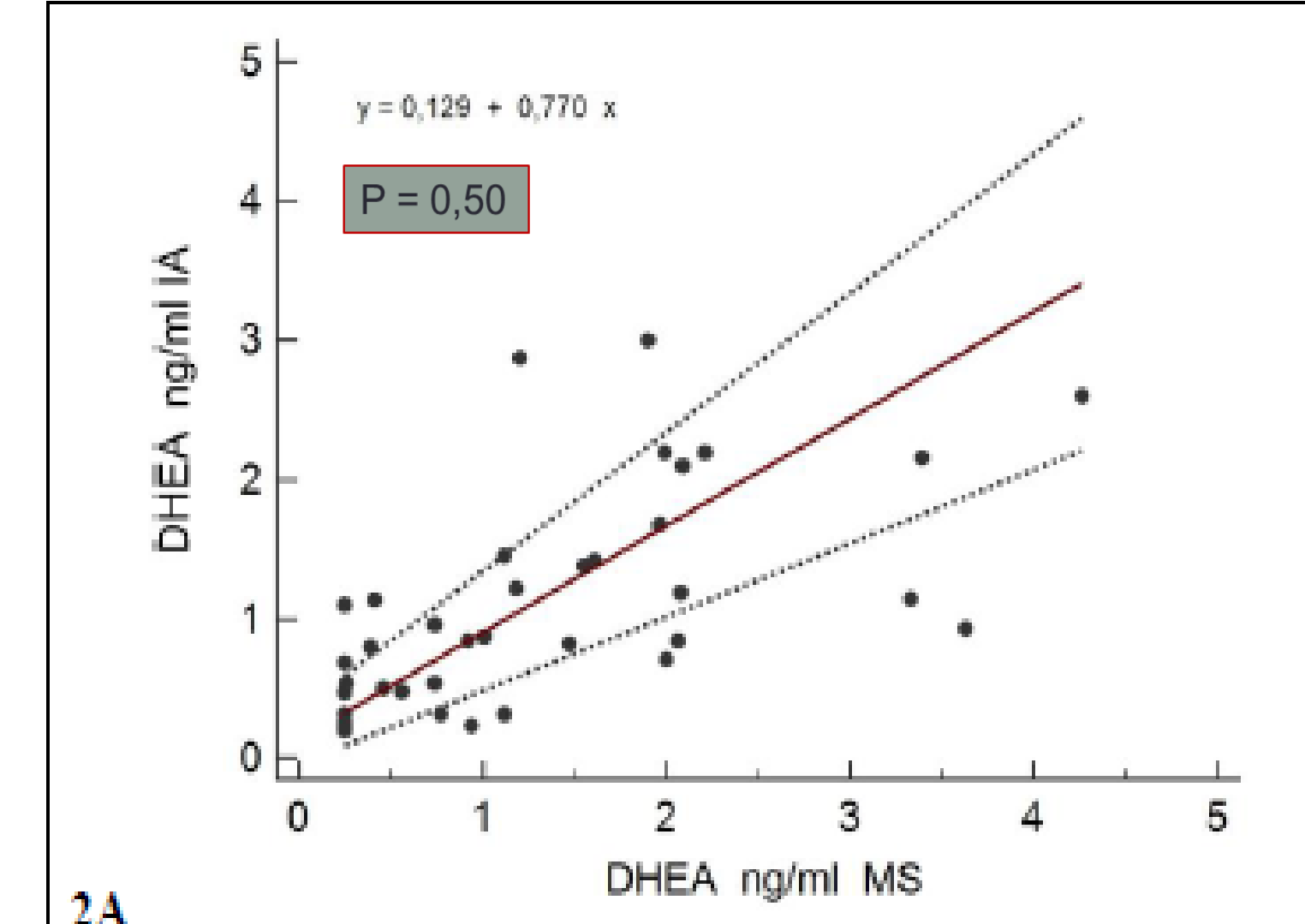
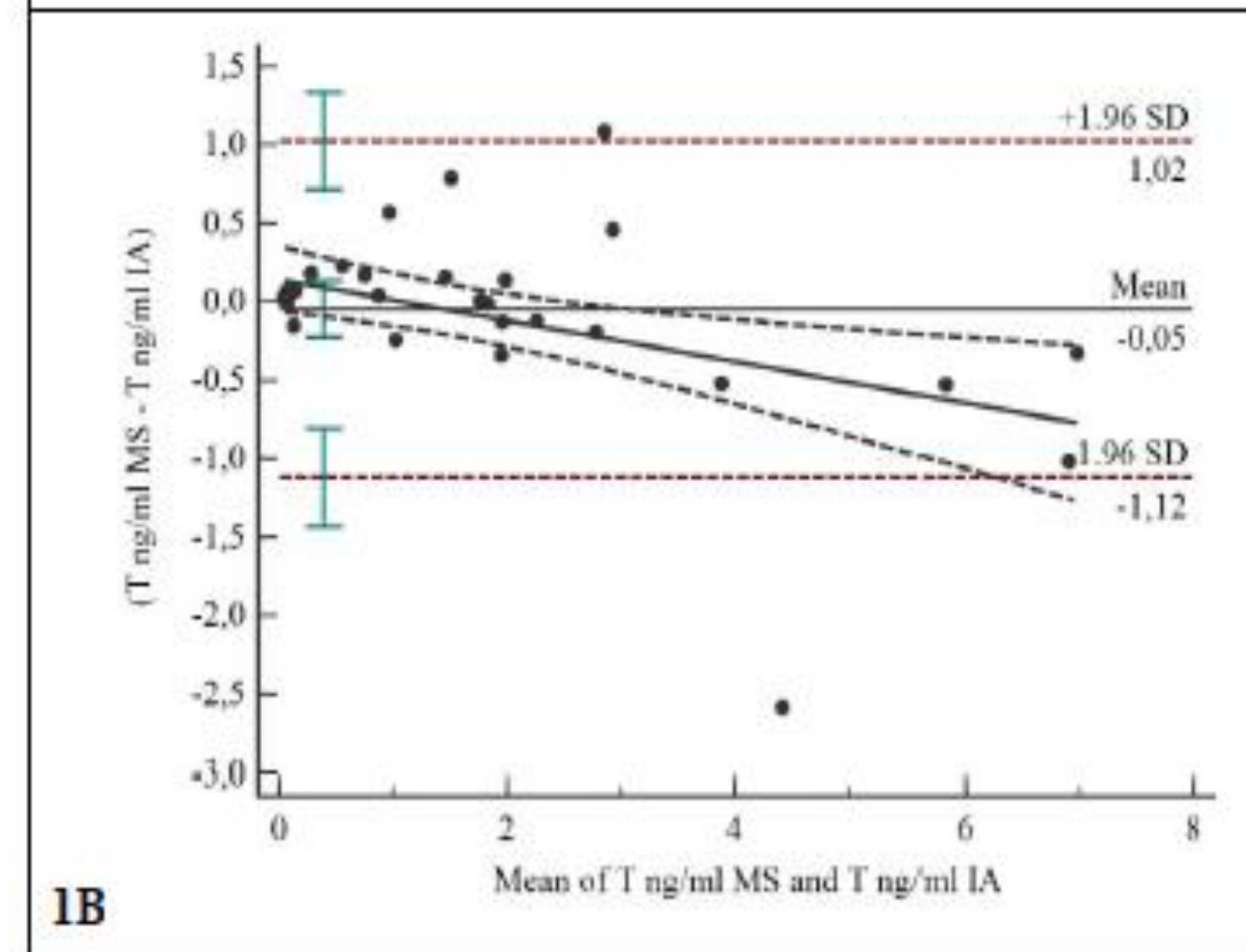
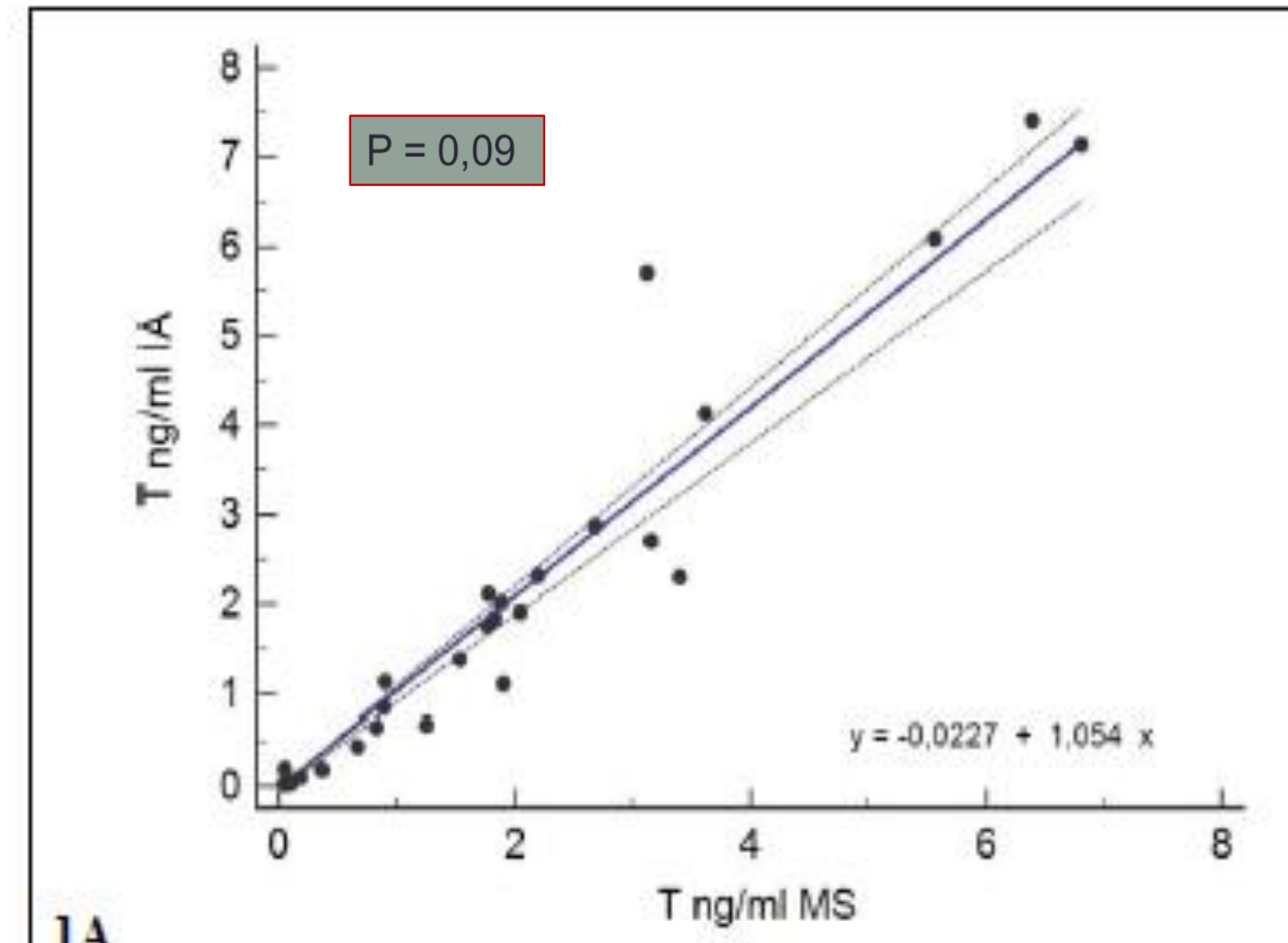
The aim of this study was to compare traditional immunoassay and LC-MS/MS methods for androgens measurements before and after recombinant human chorionic gonadotropin (rhCG) stimulation in children with 46,XY DSD.

Material and Methods

This is a prospective and cross-sectional study. We evaluated 19 prepubertal and non-gonadectomised patients: five cases of partial androgen insensitivity syndrome (PAIS), four of 5-alfa reductase type 2 deficiency and 10 of idiopathic 46,XY DSD with history of normal testosterone secretion. Total testosterone (TT), dehydroepiandrosterone (DHEA) and androstenedione (A) were measured at basal time and seven days after application of 6500 UI of rhCG. The correlation between the tests was analyzed by the Intraclass Correlation Coefficient (ICC) and Spearman Correlation Coefficient (SCC) and for the concordance analysis we used the Passing & Bablock (PB) regression and the Bland & Altman method.

Results

- There was no difference between TT, DHEA and A compared independently (Kruskal-Wallis test, $p > 0.05$)
- Values of T, A and DHEA were significantly higher after rhCG injection by both assays (Wilcoxon test, $p < 0.05$) (Table 1), with exception of DHEA by IA ($p = 0.107$).
- TT: excellent correlation in both ICC (0.958) and SCC (0.964) (Table 2), linear relationship between the values but systematic errors of concordance by PB analysis (Figure 1A). By BA method there was a tendency of the immunoassay to overestimate values towards LC-MS/MS (Figure 1B).
- DHEA: moderate correlation in both ICC (0.562) and SCC (0.716) (Table 2), linear relationship with no errors of concordance by PB analysis (Figure 2A) and tendency of IA to underestimate values towards LC-MS/MS (Figure 2B).
- A: poor correlation in ICC (0.363) and moderate in SCC (0.735) (Table 2), linear relationship but proportional and systematic errors by PB concordance analysis (Figure 3A) and tendency of IA to overestimate values towards LC-MS/MS by BA method. (Figure 3B)



✓ $y = a + bx$: linear regression equation (proportional and systematic errors)

✓ $p > 0,05$: linear relationship

✓ Mean: average difference between both assays values

A: PB regression
B: Bland-Altman graphic
T: Testosterone
DHEA: Dehydroepiandrosterone
A: Androstenedione

Figures 1-3 – Concordance analysis between IA and LC-MS/MS: PB and BA

References

1. Honowr JW. Steroid Assays in Paediatric Endocrinology. J Clin Res Ped.2010;2(1):1-16
2. Handelsman DJ, Wartofsky L. Requirement for mass spectrometry sex steroid assays in the Journal of Clinical Endocrinology and Metabolism. J Clin Endocrinol Metab. 2013;98(10):3971-3
3. Mukaka MM. A guide to appropriate use of correlation coefficient in medical research. Malawi Med J.2012;24(3):69-71

Table 1- Mean values of hormones before and after rhCG

| Time | TT (IA) | | TT (MS) | | DHEA (IA) | | DHEA (MS) | | A (IA) | | A (MS) | |
|------|---------|------|---------|------|-----------|------|-----------|------|--------|------|--------|------|
| | Basal | Post | Basal | Post | Basal | Post | Basal | Post | Basal | Post | Basal | Post |
| Mean | 0.22 | 2.81 | 0.27 | 2.66 | 1.03 | 1.13 | 0.75 | 1.84 | 0.43 | 0.54 | 0.13 | 0.21 |

Table 2- Values of Correlation Analysis between IA and LC-MS/MS

| | TT | DHEA | A |
|-----|---------------------------------|---------------------------------|---------------------------------|
| ICC | 0.958 (IC95%: 0.921 – 0.978) | 0.562 (IC95%: 0.304 – 0.704) | 0.363 (IC95%: 0.058 – 0.606) |
| SCC | $r = 0.964$; $p < 0.001$ | $r = 0.716$; $p < 0.001$ | $r = 0.735$; $p < 0.001$ |

IC: confidence interval; r: correlation coefficient; p: p value

Conclusion

The values of LC-MS/MS method and IA have a good correlation and are linearly related but the concordance analysis pointed systematic and proportional errors, with the IA overestimating TT and A values and underestimating those of DHEA in relation to LC-MS/MS. These results indicate a constitutive difference between the two evaluated assays, so IA should be carefully used when performing steroid measurements.