Congenital Adrenal Hyperplasia Newborn Screening: Improving the Effectiveness of the Neonatal 17OH-Progesterone and Serum Confirmatory Tests

Carvalho DF[1], Hayashi GY[1,2], Miranda MC[1], Valassi HP[1], Alves AN[1], Rodrigues AS[1], Gomes LG[1], Madureira G[1], Mendonca BB[1], Bachega TASS[1]

(1) Laboratório de Hormônios e Genética Molecular LIM42, Disciplina de Endocrinologia, Hospital das Clínicas, Faculdade de Medicina da Universidade de São Paulo, São Paulo/SP, Brazil; (2) Laboratório da APAE SÃO PAULO, São Paulo/SP, Brazil

Introduction and Objectives

Main concerns of CAH-NBS are the high rate of false-positive results (FPR), low positive predictive value (PPV) and heterogeneity of confirmatory tests´ methodologies. Considering the CAH-NBS implementation in our country, our **Objectives** are to optimize the neonatal 17OHP (N17OHP) cutoffs and to evaluate the performance of serum confirmatory tests.

Patients and Methods

- Samples from 473,983 newborns: N17OHP was measured by IFMA (*AutoDelfia*) and cutoffs (99th and 99.8th) were adjusted to birth-weight (BW1:<1500g; BW2:1500-2000g; BW3: 2001-2500g; BW4:>2500g), and to age at sample collection (before/after 72hs of life).
- For serum confirmatory tests, 17OHP (radioimmunoassay and liquid chromatography with mass spectrometry) and 21-deoxicortisol (21DF), Δ4 and cortisol (LC-MS/MS) were analyzed.
- Asymptomatic newborns with persistently increased 17OHP levels had the *CYP21A2* gene sequenced.

Results

- The recall rate was 0.05% using the P99th of N17OHP levels and 0.03% using the P99.8th; consequently, PPV increased from 11% to 17%.
- Considering that N17OHP cutoffs in samples collected earlier (<72hs) were significantly lower than those collected later, different N17OHP cutoffs according to BW and age were determined.
- Serum confirmatory tests were performed in 149 newborns and FPR persisted in 70% using RIA and 13% using LC-MS/MS; PPV of LC-MS/MS methodology was significantly higher than RIA (52 vs. 27%).
- Serum 21DF and steroid ratios [17OHP/cortisol; (17OHP+Δ4)/cortisol; (17OHP+21DF)/cortisol] presented similar FPR and PPV values in comparison to 17OHP by LC-MS/MS.
- 26 (22 SW/12 males) newborns presented with classical forms of CAH, confirmed by molecular analysis.
- Among 28 asymptomatic newborns with persistently increased serum 17OHP levels, genotype identified 2 NC males. The remaining were discharged from follow-up.

Table 1 – Comparison between RIA and LC-MS/MS methodologies for serum 17OHP dosages

Serum 17OHP (RV < 2.0 ng/ml)	RIA	LC-MS
Sensitivity (%)	100	100
Specificity (%)	**15.7**	**86.7**
Positive Predictive Value-PPV (%)	**27.1**	**52**
Negative Predictive Value-NPV (%)	100	100

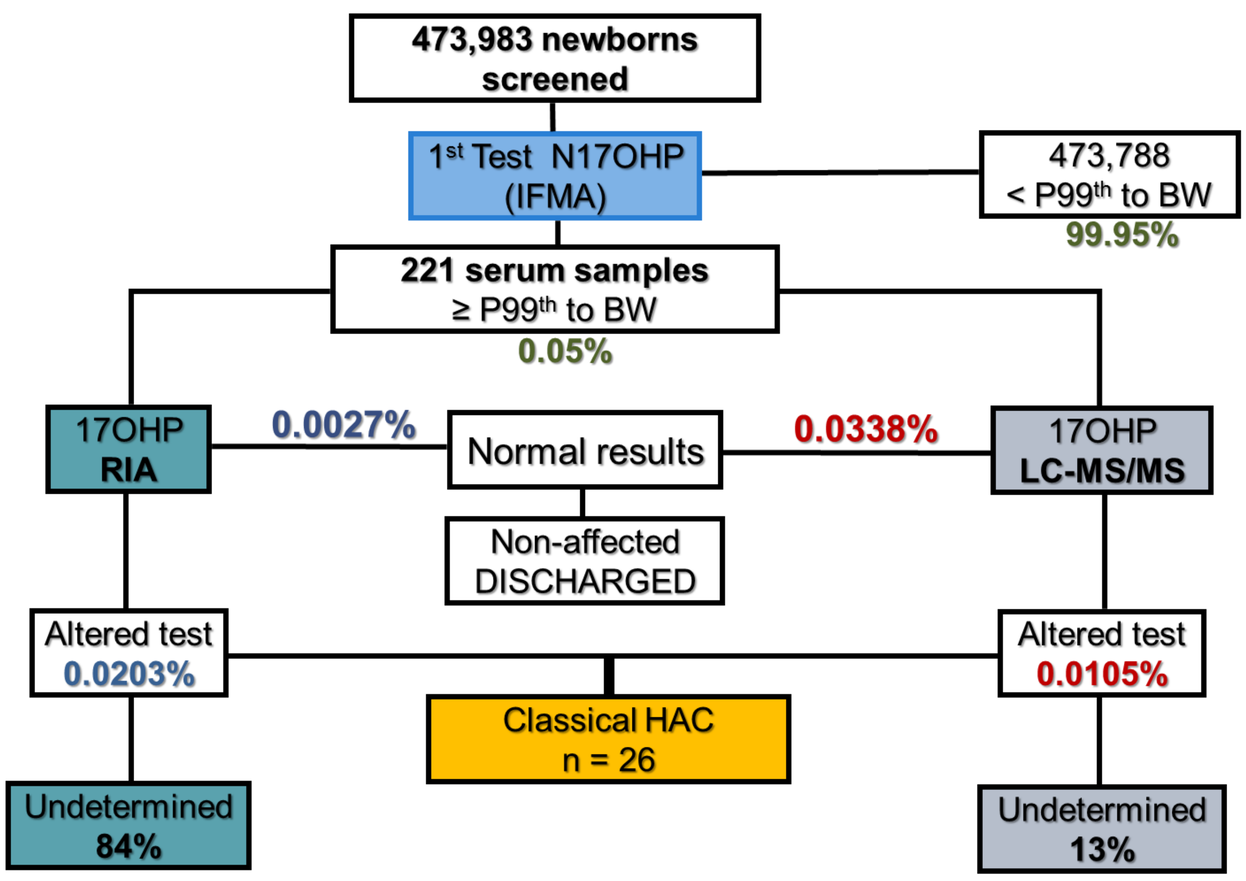

Figure 1 – Results of CAH-NBS according to different methodologies. Rates are expressed in % of the 473,983 newborns.

Conclusions

- Neonatal 17OHP levels adjusted to P99.8th according to birth-weight and age at sample collection improve the CAH-NBS by reducing the FPR rate without missing the classical form diagnosis.
- Although serum 17OHP by RIA is widely used as confirmatory test in our country, 17OHP dosage by LC-MS/MS significantly reduced recall rate.
- The 21DF and steroid ratio measurements did not provide higher accuracy than serum 17OHP by LC-MS/MS.
- Molecular analysis could be restricted for asymptomatic newborns with persistently increased 17OHP levels.

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Daniel Carvalho

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