Comparison of Newborn Screening Protocols for Congenital Adrenal Hyperplasia in Preterm Infants

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Objective To compare 2 screening protocols performed concurrently in Minnesota: (1) liquid chromatography tandem mass spectrometry steroid profiling as a second-tier test on positive fluoroimmunoassay (FIA) results; and (2) low-birthweight 3-screen protocol (FIA tests at <48 hours, 2 weeks, 4 weeks) on all infants <1800 g, regardless of result.

Study design Population-based study of all <1800 g infants (n = 8739) born in Minnesota from 2004-2010 comparing newborn screening performance metrics of 2-tier (FIA + liquid chromatography tandem mass spectrometry) protocol (2004-2010) vs 1-tier (FIA) low-birthweight 3-screen protocol (2006-2010). False positive (FP) rates were calculated per infant's final confirmatory result. Protocol results used in different time periods (2004-2005 vs 2006-2010) were compared by 2-sample tests of proportions; results of both protocols for 2006-2010 were compared by McNemar test.

Results First-tier testing of final dried blood spot result (n = 6625) of the low-birthweight 3-screen protocol during 2006-2010 reduced the FP rate more than 5-fold ($P < .0001$) compared with 2-tier testing of a single dried blood spot (n = 2114) from 2004-2005. In comparing results (n = 6625) of both protocols from 2006-2010, first-tier testing of final dried blood spot accounted for 23% of FPs; second-tier testing of the first dried blood spot accounted for 77%, yielding significantly more FP results (McNemar test, $P < .0001$).

Conclusion Timing of dried blood spot collection rather than assay used played a more important role in reducing FP results of congenital adrenal hyperplasia newborn screening in low birthweight infants. *(J Pediatr 2014;164:1136-40)*.

The purpose of newborn screening (NBS) for congenital adrenal hyperplasia (CAH) due to 21-hydroxylase deficiency is the early identification of newborns with the classic salt-wasting and simple virilizing forms to avoid a potentially life-threatening adrenal or salt-wasting crisis. Most NBS programs following a 1-tier protocol use a time-resolved fluoroimmunoassay (FIA) to measure 17-hydroxyprogesterone (17OHP) from dried blood spots on filter paper. Although NBS identifies over 200 children each year affected with CAH in the US, high false positive rate (FPRs) among preterm infants continue to plague screening programs and neonatal intensive care units (NICUs). The challenge in screening preterm infants for CAH is that transient elevated 17OHP levels, because of birth stress, adrenal immaturity, and early screen collection, lead to high FPR.[1-3] Adjusted 17OHP cutoff values by birthweight and/or gestational age have been implemented, but have not been successful in significantly reducing the FPR in the preterm population.[4,5]

In June 2004, the Minnesota NBS program implemented a 2-tier protocol by adding second-tier testing for CAH using liquid chromatography-tandem mass spectrometry (LC-MS/MS) to measure 17OHP, androstenedione, and cortisol on those samples that had elevated 17OHP first-tier results.

Two years later, in January 2006, Minnesota implemented a low birthweight 3-screen protocol for screening infants weighing ≤1800 g. This protocol requires NICU and Special Care Nursery facilities within the state to draw 3 screens on all inpatient infants ≤1800 g (24-48 hours, 2 weeks, and 4 weeks/or discharge). This 3-screen algorithm was performed in conjunction with the 2-tier protocol.

17OHP	17-hydroxyprogesterone
CAH	Congenital adrenal hyperplasia
FIA	Fluoroimmunoassay
FN	False negative
FP	False positive
FPR	False positive rate
LC-MS/MS	Liquid chromatography-tandem mass spectrometry
NBS	Newborn screening
NICU	Neonatal intensive care unit
PPV	Positive predictive value
TP	True positive

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The authors declare no conflicts of interest.

0022-3476/$ - see front matter. http://dx.doi.org/10.1016/j.jpeds.2014.01.038

Using results from preterm infants (≤1800 g) from 2004-2010, we compared FPR and positive predictive value (PPV) of the 2 testing protocols. Drawing on 6.5 years of experience, this study examines the NBS performance metrics for CAH in premature infants of both second-tier steroid profiling and a NICU 3-screen system.

Methods

All premature infants ≤1800 g (n = 8739) screened in the state of Minnesota from June 2004 through December 2010, with the majority being of Western European descent were included. NBS results were documented by the short-term NBS follow-up program of the Minnesota Department of Health. Institutional Review Boards at all sites approved the study.

First-Tier FIA

All first-tier 17OHP CAH screens used whole blood drawn by heel prick or venous puncture and dried on filter paper collected over a 1-month period after birth. For determination of 17OHP, a time-resolved FIA (DELFIA; PerkinElmer Life and Analytical Sciences, Turku, Finland) that utilizes weight-based cutoff values[6,7] was used. First-tier 17OHP cutoff values were adjusted during the study period according to the assay and antibody used. For more detail about changes in 17OHP cutoff values, assay and antibody used please refer to Sarafoglou et al.[8]

Second-Tier Steroid Profiling (LC-MS/MS)

After June 1, 2004, a first-tier positive result was flagged and a sample from the original dried blood spot was automatically analyzed at Mayo Clinic's Biochemical Genetics Laboratory using a second-tier method of steroid profiling by LC-MS/MS as described in Lacey et al.[9] Initial reference ranges set by Mayo Clinic were lowered in February 2006.

CAH 2-Tier Screening Protocol (FIA and LC-MS/MS)

In the 2-tier protocol, if a first-tier result was negative, the result was classified as negative. If the first-tier result was positive and the second-tier result (either normal 17OHP concentration or normal 17OHP + androstenedione divided by cortisol ratio) was negative, the result was classified as negative. If the second-tier was positive (typically both the 17OHP level and ratio were elevated), the result was presumed positive, but was not classified as true positive (TP) or false positive (FP) until the diagnosis was confirmed by an endocrinologist.[8,10]

CAH NICU 3-Screen Protocol (FIA)

In January 2006, the Minnesota NBS Program recommended that NICU and Special Care Nursery facilities within the state draw 3 newborn screens on all ≤1800 g infants over a period of 1 month (24-48 hours, 2 weeks, 4 weeks/or discharge) to capture premature infants with initial false negative (FN) newborn screens for congenital hypothyroidism because of delayed thyrotropin elevations.[11,12] Minnesota's Low Birth Weight Protocol Workgroup, comprising endocrinologists and neonatologists, chose ≤1800 g as the cutoff, rather than the customary 2000-2500 g, to reduce the number of infants unnecessarily undergoing the 3-screen protocol. Because Minnesota's NBS program does not differentiate repeat screens from initial screens, all analytes, not just thyrotropin, were also screened 3 times, including 17OHP measured by first-tier FIA. Per protocol, 3 screens were requested from each premature infant (≤1800 g) whether or not the screens were normal. Second-tier steroid profiling was performed only on those samples that had elevated 17OHP first-tier results. The low birthweight 3-screen protocol was performed in conjunction with the 2-tier steroid profiling protocol. An infant with an abnormal first or second NBS screen would only be referred by the neonatologist for diagnostic testing (serum 17OHP level and endocrine evaluation) if there was symptomatology suggestive of CAH (abnormal electrolytes, ambiguous genitalia).

Statistical Analyses

We have used the final diagnostic determination after confirmatory testing by an endocrinologist to classify the NBS result for each infant as TP, true negative (TN), FP, or FN. All rates were calculated per infant's final confirmatory result. Accuracy of screening results were assessed with the FN rate FNR = FN/(TP + FN) = (1 − sensitivity), FPR = FP/(TN + FP) = (1 − specificity), PPV of the screen PPV = TP/(TP + FP), and detection rate = average number that need to be screened to detect 1 case = 1: (number screened/TP).

We compared the accuracy of screening results from first-tier testing of the final dried blood spot (2006-2010) with 2 other testing results: (1) all screening results from the preceding period (2004-2005), an independent sample; and (2) from 2006-2010, screening results from second-tier (LC-MS/MS) applied to the first dried blood spot in the same infants (a paired sample). Results from independent samples from different time-periods (2004-2005 vs 2006-2010) were compared using 2-sample tests of proportions with exact CIs. Paired results from 2 different methods applied to the same infants from 2006-2010 were compared by McNemar test for paired binary observations, which compares rates of positive results; exact 95% CIs are given for FPR and PPV, but because of the pairing, these values are not independent and there is no significance test.

To assess mean change in 17OHP during weeks after birth, 17OHP was transformed to the log scale for analysis. For each infant, the first measurement of 17OHP taken in each screening interval after birth (0-4 days, 7-21 days, 21 + days) was used, so that each infant contributed up to 3 measurements. The 3 screens were compared within each birthweight class using a mixed effects linear model with fixed effects for the screen, very low birthweight status, the screen, very low birthweight interaction, and a random intercept for each infant to model the within-infant correlation between repeated measurements. Back-transformed