Stopping Parenteral Nutrition for 3 Hours Reduces False Positives in Newborn Screening

Thipwimol Tim-Aroon, MD[1,2], Heidi M. Harmon, MD[3], Mary L. Nock, MD[3], Sreekanth K. Viswanathan, MD[3], and Shawn E. McCandless, MD[1,2]

Objective To evaluate effects of holding parenteral nutrition (PN) for 3 hours prior to newborn screening (NBS) on false-positive NBS rate for amino acids (AAs) in very low birth weight (VLBW) infants (birth weight <1500 g).

Study design We analyzed data from 12 567 consecutive births in 1 hospital between May 2010 and June 2013. VLBW infants were stratified into 3 groups: (1) infants without PN before NBS (no-PN group); (2) infants with early PN running at the time of NBS (early-PN group); and (3) infants with early-PN that were temporarily replaced by dextrose-containing intravenous fluid 3 hours prior to NBS (stop-PN group). We compared the false-positive rate for AA and cost effectiveness between the groups.

Results The false-positive rate for AA among 413 VLBW infants was significantly higher than infants with birth weight >1500 g (7.62% vs 0.05%; $P < .001$). There were no false-positive results for AA in the no-PN group. The false-positive rate for AA in the stop-PN group (2/65) was significantly lower than the early-PN group (29/245) (3.1% vs 11.8%; $P = .037$). The stop-PN group was more cost effective than early-PN group, saving $17.27 per infant screened ($5.53 vs $22.80) or $192.54 for each false-positive result for AA averted. Further reductions in inconclusive samples were also noted.

Conclusions VLBW and early-PN are significant factors for false-positive results for AA. Holding PN containing AAs for 3 hours before NBS collection is a practical and cost-effective method to significantly reduce the false-positive rate for AA in VLBW infants. *(J Pediatr 2015;167:312-6)*.

Newborn screening (NBS) programs for inherited disorders were first introduced in the 1960s with massive expansion following introduction of tandem mass spectrometry in the 1990s. Like any other medical screening test, false-positive NBS have medical and economic implications, including need for additional investigations and treatments, added cost for follow-up tests and other health care utilization, and increased parental stress.[1-5]

The Ohio NBS program currently screens for at least 34 disorders. The overall false-positive rate for Ohio NBS has been approximately 1.4%.[6] Several studies have shown increased rates of false-positive NBS in preterm infants that are variously postulated to result from immature organ or endocrine function, underlying illness, transfusion, parenteral nutrition (PN), inappropriate cut-off values, and timing of NBS collection.[6-9] Over the past 15 years, most extremely premature infants are given PN containing amino acids (AAs) for prevention of catabolic state, nutritional deficiency, and growth restriction[10-12] prior to NBS blood collection at 24-48 hours of life. Recent reports demonstrate increased false-positive NBS results for hypermethioninemia in premature infants with PN,[13] and higher plasma AA concentrations in infants with early parenteral AA.[14,15] We hypothesized that holding PN containing AAs before NBS blood collection would reduce false-positive NBS rate for AAs.

Methods

This was a retrospective cohort study using the Rainbow Babies and Children's (RB&C) Hospital NBS database from May 2010 to June 2013. All infants with NBS collected by the institution and with results available in the database were included in the study population. The study, including review of the medical records, was part of a quality improvement project, evaluating a similar time period before and after institution of the new protocol. The process and data were reviewed and approved for publication by the institutional review board of University Hospitals Case Medical Center.

AA	Amino acid
BW	Birth weight
NBS	Newborn screening
NICU	Neonatal intensive care unit
PN	Parenteral nutrition
RB&C	Rainbow Babies and Children's
VLBW	Very low birth weight

From the [1]Center for Human Genetics, University Hospitals Case Medical Center; [2]Department of Genetics and Genome Sciences, and [3]Division of Neonatology, Department of Pediatrics, Rainbow Babies and Children's Hospital, Case Western Reserve University, Cleveland, OH

The authors declare no conflicts of interest.

http://dx.doi.org/10.1016/j.jpeds.2015.04.063

Early-PN with high protein (2-3 g/kg/d) started within 24 hours of birth for very low birth weight (VLBW) infants (birth weight [BW] <1500 g) has been a standard treatment in the RB&C Hospital neonatal intensive care unit (NICU) since early 2010. A new NBS collection protocol was implemented in RB&C Hospital NICU in July 2012 as part of a quality improvement initiative. As part of the new protocol, PN was held 3 hours before NBS collection and replaced with dextrose containing fluids (same dextrose concentration as previous PN) for infants <1500 g. Current Ohio NBS guidelines for VLBW infants with PN recommend initial NBS sample between 24 and 48 hours of life, or before 24 hours if the infant is to be transfused (then repeated as appropriate for metabolites that should be evaluated after protein feeding starts).

Electronic medical records of the VLBW infants were reviewed for demographic data, time when AA containing PN was initiated, time of NBS collection, and outcome of the NBS.

False-positive AA results were defined as abnormal NBS for a single AA that was not associated with an inborn error of metabolism based on follow-up test results and consultation with metabolic specialists, as clinically indicated. The Ohio NBS laboratory reports multiple elevated AAs as "inconclusive" requiring repeat NBS samples; therefore, these inconclusive results are not included in the false-positive results in the study.

The hospital charges associated with false-positive AA results, including the additional investigations, repeat NBS card, and subspecialty consultation, were ascertained from the hospital laboratory charge files and coding database. Medicaid hospital inpatient specific cost-to-charge ratios were used to adjust the hospital charges to estimate the direct costs associated with false-positive AA results (www.bwc.ohio.gov). Indirect costs that were not measured include infants' pain from the needle sticks for additional blood sampling and increased parental anxiety from the abnormal NBS result. The only additional direct cost associated with the 3-hour stop-PN was the cost of intravenous dextrose to replace PN.

Data Analyses

VLBW infants were stratified into 3 groups according to timing of the PN and NBS blood collection including: (1) no-PN group: infants without PN before NBS; (2) early-PN group: infants with early PN running at the time of NBS; and (3) stop-PN group: infants receiving early PN that was temporarily replaced by dextrose containing intravenous fluid (same dextrose concentration as PN) 3 hours prior to NBS. To determine the effect of early PN on the false-positive rate for AA in NBS, we compared the false-positive rate for AA between the early-PN and the no-PN groups. Similarly, to evaluate the effect of the new NBS collection protocol on an "intention to treat" basis, we compared the false-positive rate for AA between 2 time periods (before and after the implementation of the 3-hour stop-PN protocol).

The false-positive rate was calculated by the number of infants with false-positive results divided by total infants in that group. The χ^2 test was used for categorical variables as appropriate. A statistical test with $P \leq .05$ was considered significant. JavaStat statistical software (http://stat.wvu.edu/~jharner/projects/javastat/intro.html), was used to conduct the analysis. Hospital charges related to NBS result follow-up for patients with false-positive rate for AA were identified and averaged across the group.

Results

NBS results were available for 12 567 infants; 39 infants had positive NBS results for AAs, of which 1 infant was confirmed as a true positive result for maple syrup urine disease. The remaining 38 infants had false-positive results. The false-positive rate for AA in the entire study population was 0.3% (38/12 567). The false-positive rate for AA in VLBW infants (N = 420) was significantly higher (7.62% vs 0.05%; $P < .001$) than in infants with BW more than 1500 g (N = 12 154), thus, VLBW infants accounted for 3.3% of the study population (420/12 567), but 89% (32/38) of the false-positive AA results. Furthermore, 63% (20/32) of the VLBW infants with false-positive NBS for AA results were extremely low BW (<1000 g).

The single false-positive AAs in VLBW infants are shown in **Table I** by the abnormal metabolite. All false-positive AA results had only modestly increased concentrations of the AA above the NBS laboratory's cut-off value.

The false-positive rates for AAs in VLBW infants before implementation of the new "stop-PN " policy compared with after implementation were not significantly different, 7.9% (24/301) and 6.7% (8/119). This was explained after manual inspection of the medical records, both electronic and paper, revealed that the 3-hour stop-PN protocol had only been followed in 54% of VLBW infants (64/119) after the protocol was initiated in July 2010. As with most new protocols in this large NICU, with many dozens of nursing providers, the actual implementation of the protocol

Table I. AA levels from NBS in infants with false-positive AA results

Mean concentration umol/L	BW <1500 g (range) (n = 32)	BW ≥1500 g (range) (n = 6)	Ohio NBS cut-off levels
Methionine	126 (100-248) (n = 19)	122 (n = 1)	<100
Phenylalanine	165.6 (160-173) (n = 5)	162.5 (61-164) (n = 2)	<160
Tyrosine	509 (438-585) (n = 5)	475 (406-527) (n = 3)	<400
Arginine	113 (101-124) (n = 3)	-	<100