Reduction in Newborn Screening False Positive Results Following a New Collection Protocol: a Quality Improvement Project

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OBJECTIVE Premature infants are known to have a higher rate of false positive newborn screening (NBS) results, with TPN as a contributing factor. The purpose of this quality improvement (QI) project is to reduce false positive NBS results via a TPN interruption protocol

METHODS A multidisciplinary team reviewed the literature and developed a new NBS collection protocol, which was implemented in 2 periods. In period 1, TPN was interrupted for 4 hours before NBS sample collection and initiation of carnitine supplements was avoided. In period 2, TPN was interrupted for 6 hours for infants birth weight (BW) < 1000 g, carnitine supplementation continued to be avoided. The rates of false positives NBS results were compared pre- and post-interventions in periods 1 and 2.

RESULTS Four hundred twelve neonates were evaluated prior to implementation of this QI project (July 2013–June 2014) and 414 during period 1 intervention (July 2014–June 2016). False positive results decreased from 20.6% to 11.4% ($p < 0.001$) among all BW categories following the 4-hour TPN interruption. The rate of false positives was further reduced among infants < 1000 g ($p = 0.035$) in period 2 ($n = 112$), including a significant reduction in false positive results with elevated amino acid profiles ($p = 0.005$).

CONCLUSIONS The implementation of a strict NBS collection protocol reduced false positive NBS results, which potentially can improve patient care by reducing unnecessary laboratory draws, pain, and parental anxiety. Interruption of TPN for 6 hours was significant in reducing NBS false positive results in neonates < 1000 g.

ABBREVIATIONS AAP, American Academy of Pediatrics; BW, birth weight; ELBW, extremely low birth weight; NBS, newborn screening; NICU, neonatal intensive care unit; QI, quality improvement; TPN, total parenteral nutrition; VLBW, very low birth weight.

KEYWORDS false positive; neonatal intensive care; newborn screening; quality improvement; total parenteral nutrition

J Pediatr Pharmacol Ther 2021;26(7):723–727

DOI: 10.5863/1551-6776-26.7.723

Introduction

Newborn screening (NBS) is a routine part of infant care, allowing for early detection of endocrine, metabolic, and other genetic diseases, even if asymptomatic, and prompt initiation of therapy. The AAP and the American College of Medical Genetics and Genomics recommend that all newborn infants undergo NBS shortly after birth.^{1,2} The number of diseases screened for has greatly increased over the years with the availability of technology and using only a few drops of blood. In 2006, the Recommended Universal Screening Panel in the United States was expanded to include 29 core conditions, including several amino acidemias and fatty acid oxidation disorders screened for via tandem mass spectroscopy.³ As of July 2018, all 50 states have implemented screening for at least 29 of the 35 core conditions recommended by the current Recommended

Universal Screening Panel, with some states offering NBS for 40 to 50 inherited diseases.⁴

Premature infants or those requiring additional care in the NICU are at higher risk for false positive NBS results.^{3,5,6} 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.^{7,8} Previous research also suggests that parents experience stress and anxiety when their child is retested after a positive NBS and negative effects may persist even when the result is a false positive.^{8,9}

In the NICU, there have been several factors associated with false positive NBS.^{5,10-12} In particular, TPN provided in the NICU and the liver immaturity of preterm infants have been recognized as major contributors to the high false positive NBS rates. TPN supplies fundamental nutritional requirements to preterm infants in the NICU. The standard of care in neonatal settings is

to provide early nutrition support via initiation of TPN to promote growth and development.¹³ However, infants on TPN may have NBS results with multiple minor amino acid abnormalities,¹⁴ increasing false positive NBS results. In these cases, follow-up screening has been recommended when the infant is off of TPN. Recently, researchers investigated whether withholding TPN for 3 hours before NBS blood collection would reduce false positive results.^{13,15} This protocol resulted in a reduced false positive NBS rate and reduced costs associated with the confirmatory testing for false positives by >80%.¹³ However, the results were inconsistent among very low birth weight (VLBW) infants (1000–1500 g) and extremely low birth weight (ELBW) infants (<1000 g). These groups often have a higher false positive rate compared with other birth weight (BW) groups (>1500 g). For instance, Asghar et al¹⁶ recently reported that among infants with BW < 1000 g, false positive NBS results were found among 70% of cases and among half of screened VLBW infants. Thus, further efforts to reduce rates of false positive NBS results in VLBW and ELBW infants are still needed.

This quality improvement (QI) project was conducted with the aim of reducing false positive NBS results including an abnormal acylcarnitine profile, elevated amino acids, or combination of both via implementing a new NICU protocol of withholding TPN for up to 6 hours.

Methods

This retrospective study was part of a QI project evaluating false positive NBS results before and after implementation of a new NBS protocol at Sanford Health's Level III NICU. Data were collected on all infants born at Sanford Health's Medical Center in Fargo, ND or admitted to the NICU within 24 hours of life from July 2013 to June 2019 using the NICU's electronic medical record system. This QI project was approved by the Quality Council of our NICU, a committee responsible for quality initiatives and ensuring quality of care. Information including BW, NBS final reports, and diagnostic testing results for NBS abnormalities were extracted. Positive NBS results were defined as elevated amino acids, abnormal acylcarnitine profiles, or combination of both. Each positive result on any NBS sample was tracked until its final resolution. Final resolution was accomplished by repeat NBS collection. Infants were excluded from the analysis if the NBS was a true positive or if any of the following information was incomplete: final NBS report, results of diagnostic testing performed in response to an abnormal NBS, or documentation of final diagnosis for NBS abnormality.

Prior to this QI project, NBS blood samples at our institution were collected without TPN interruption. A multidisciplinary QI team (including a neonatologist, pharmacist, nurse practitioner, nurse educator, and nurse manager) reviewed the literature and developed a new newborn screen collection protocol. The QI

project was conducted in 2 data collection periods. In the first period (July 2014–June 2016), TPN was interrupted for 4 hours while infusing dextrose 10% in water at the same rate as the TPN. Initiation of carnitine supplements in TPN was avoided during this intervention period until NBS results were back. Collection of NBS samples occurred via a single heel-stick by NICU nursing staff. After the specimen was collected, the TPN was restarted. Site, time, interruption of TPN, and avoidance of carnitine were tracked for every newborn screen sample to ensure compliance. The rates of false positives NBS results were compared pre- and post-intervention. Pre-intervention consisted of a 1-year (July 2013–June 2014) prior to implementing TPN interruption protocol for NBS. During the pre-intervention period, NBS samples were collected from all infants regardless of TPN administration.

In the second period (August 2016–June 2019), the NBS collection protocol was further modified for infants with BW < 1000 g in an effort to decrease false positive results and TPN was interrupted for 6 hours. All other interventions remained the same including collection technique and avoidance of carnitine. Results from this period were compared with those from the first period (TPN interruption for 4 hours). A comprehensive nursing/medical staff educational initiative was conducted to improve techniques for obtaining samples in each study period and to provide an overview of the changes to the NBS guideline.

For both the first study period and second study period, an admission order set was used with pre-populated instructions on how to collect the NBS. The instructions were put both on the order to obtain the NBS and on the dextrose 10% order. For example, the dextrose order during period 2 reads, "To run in place of amino acid containing fluid at the same rate 4 hours prior to newborn metabolic screen drawn OR 6 hours prior to screen if infant is less than 1000 gm." These instructions were added to help prompt both the nurse and provider to run the dextrose 10%. Additionally, a best practice alert was added in the EPIC system if a provider tried to add carnitine in the TPN or as an enteral supplement prior to 7 days of life. The alert was to remind providers that carnitine may alter the results of the NBS and result in false positives. A provider may override and document this alert if the benefits of providing the supplement outweigh the risk.

After collection, the NBS was then sent to the University of Iowa State Hygienic Laboratory in Ankeny, IA for processing. Follow-up testing was then recommended and coordinated with the North Dakota Department of Health and with the Iowa Children's Hospital. Recommendations after a possible positive screen varies, however, may include repeat testing once a patient is off TPN or has undergone a certain period of time without other interventions such as a

Table 1. Newborn Screening Test Results Total and By Birth Weight Category for Pre-Intervention Versus Study Period 1 (4-hr TPN Interruption; July 2013–June 2014)

Birth Weight, g	Pre-intervention		Period 1 (4-hr TPN Interruption)		p value
	n	False Positive, n (%)	n	False Positive, n (%)	
<1000	26	18 (69.2)	35	15 (42.9)	0.058
1000–1500	37	24 (64.9)	28	7 (25.0)	0.001*
>1500	349	43 (12.3)	351	25 (7.1)	0.020*
Total	412	85 (20.6)	414	47 (11.4)	<0.001*

* p value significant at 0.05.

Table 2. Newborn Screening Test Results for Infants <1000 g Comparing the Two Study Periods

Period	PI (n = 26)	Period 1 (n = 35)	p value (PI vs Period 1)	Period 2 (n = 51)	p value (Period 1 vs. Period 2)
Total false positive rate, n (%)	18 (69.2)	15 (42.9)	0.058	11 (21.6)	0.035*
Breakdown of false positives, n (%)					
Abnormal acylcarnitine	1 (3.8)	2 (5.7)	0.741	5 (9.8)	0.497
Elevated aa	12 (46.2)	13 (37.1)	0.477	6 (11.8)	0.005*
Combination	5 (19.2)	0 (0)	0.007*	0 (0)	<0.001*

aa, amino acid; PI, Pre-intervention

* p value significant at 0.05.

blood transfusion.

Infant data were stratified into 3 groups according to the timing of TPN and NBS blood collection including: 1) those pre-intervention without TPN interruption; 2) those with 4-hour TPN interruption; and 3) those with 6-hour TPN interruption among BW < 1000 g infants. In Period 1, the false positive rate was compared for all BW groups (<1000 g; 1000–1500 g; >1500 g) for pre- and post-intervention data available for 4-hour TPN interruption. Negative newborn screens were assumed true negative. In period 2, the rate of false positive NBS between the 2 study periods (4-hour versus 6-hour interruption) was also compared for infants with BW <1000 g. The false positive rate was calculated by the number of infants with false-positive results divided by total infants in that group. Among infants with BW < 1000 g, elevated amino acid and abnormal acylcarnitine levels were also compared for the 2 interruption periods. The χ^2 test was used to compare the differences in the false positive rate between periods, stratified by BW categories or broken down by diagnostic results in period 2. For subgroup analysis, when the assumptions of a χ^2 test were not met due to small sample size, the 2-sided Fisher exact test was used. Relative risk of a false positive result was calculated periods 1 and 2 among neonates with BW < 1000 g. Data analysis was conducted on SPSS (SPSS, Chicago, IL) and $p \leq 0.05$ was considered significant.

Results

Out of 868 infants meeting the inclusion criteria, there was a total of 851 NBS samples included in the analysis over the study period. The remaining 17 were excluded due to loss to follow-up if transferred to another facility post birth or due to mortality.

Pre-intervention. During the 1-year pre-intervention, 412 infants among the 3 BW groups were screened and included in the analysis. All infants who were screened and met the inclusion criteria were included in the pre-intervention sample. The total false positive rate was 20.6% with over 50% of infants with BW <1500g having a false positive test result.

Period 1. A total of 414 infants were included in the analysis of period 1 (Table 1). Overall compliance rate of the protocol was 95%. Carnitine was avoided in all patients prior to NBS collection. Across all groups, there was a significant reduction in the number of false positive results with the 4-hour TPN interruption (20.6% vs 11.4%, $p < 0.001$). Analysis by BW category showed that the new protocol reduced the false positive rate (FPR) significantly in infants with BW > 1000 g. The reduction was greatest in infants with a BW 1000 to 1500 g (39.9%, $p = 0.001$). Because the reduction in the false positive rate among ELBW neonates was not significant, a 6-hour TPN interruption protocol was implemented in period 2.

Period 2. Data for period 2 included a total of 112 infants with BW < 1000 g. Overall compliance rate of the

protocol was 98%. The modified NBS protocol with TPN interruption prolonged to 6 hours further reduced the false positive rate by 21.6% ($p = 0.035$) when comparing period 1 data as shown in Table 2. Carnitine was avoided prior to the NBS being drawn in all patients. Further, relative risk among infants with < 1000 g BW for a false positive result was reduced from 0.5 (95% CI, 0.28–1.03) with the 4-hour intervention to 0.4 (95% CI, 0.22–0.71) in period 2 ($p = 0.0004$). Analyses of NBS abnormalities for each of the study groups revealed that the number of positives for elevated amino acids was reduced from 46.2% ($n = 12/26$) to 37.1% ($n = 13/35$) to 11.8% ($n = 6/51$) when comparing prior to implementation, period 1 and period 2, respectively, with a significant reduction in period 2 ($p = 0.005$). However, the new protocol did not significantly reduce the number of infants with abnormal acylcarnitine levels on false positive NBS results ($p = 0.497$). Those that had a false positive abnormal acylcarnitine profile did not have carnitine added to the TPN prior to the NBS being drawn.

Discussion

False positive NBS results are a major concern for NBS programs. Following modifications to the NBS protocol, our results indicate a significant reduction in the false positive rate for NBS results amongst all BW groups. The protocol was effective in reducing FP NBS with no known false negative results.

The highest false positive rate pre-intervention (69%) was seen in infants with a BW < 1000 g when compared with the other BW groups. This population almost always has exposure to amino acids in a starter TPN or in a custom TPN early in life, whereas patients in other weight cutoffs may or may not qualify for TPN. This is consistent with previous findings.^{13–15} Several previous reports have examined new methods, protocols, or cutoff values to reduce false-positive NBS rates.^{4,14,17} Research that examined the value in TPN interruption has focused on a 3-hour stop in TPN in effort to reduce the false positive rate for NBS.^{13,15} Although those results reported a positive reduction in the false positive rate, this was minimal for the < 1000 g BW group. This study shows a new NBS collection protocol with a 4-hour and a 6-hour TPN interruption, both of which resulted in a greater reduction in the false positive rate of NBS among infants than previously reported. For instance, in Morris et al,¹³ interrupting TPN for 3 hours prior to NBS sample collection resulted in a 4% reduction in the false positive rate for infants with 1000 to 1500 g BW, whereas reduction in the rate in our study was 40% for that BW group with 4 hours of TPN interruption. A dose-response relationship was also observed with longer TPN interruption in period 2 resulting in a lower false positive rate among infants with BW < 1000 g.

Infusion of amino acids has been reported to increase

the NBS false positive rate.¹⁸ Our protocol in period 2 was successful in reducing NBS false positive results that reported elevated amino acid abnormalities. However, abnormal acylcarnitine profiles were not reduced with the introduction of the 6-hour TPN interruption. This may be attributed to the fact that carnitine infusion practice did not change between periods 1 and 2. No patients in study period 1 and 2 received carnitine prior to the NBS collection. Methods to reduce abnormal *acylcarnitine* profiles in false positive NBS are thus still needed.

In our NICU, the initial starter TPN comprised dextrose 10% and amino acid solution (Trophamine) 5% with an initial rate of 60 mL/kg/day. This provides the patient with an initial glucose infusion rate of 4.2 mg/kg/min and 3 g/kg/day of protein. When TPN is interrupted for 6 hours using just a D10 solution, the patient still will get at least 2.25 g/kg/day from the remaining 18 hours of TPN. Most patients have a custom TPN at the time the newborn screen is drawn though, which often contains even higher amounts of protein. Typically, protein is advanced to a goal of 3.5 to 4 g/kg/day over the first 72 hours of life with the custom TPN. The current recommendations are 3 to 4 g/kg/day for preterm infants.¹⁹ We also initiate a mixed lipid emulsion within the first 24 hours of life at a dose of 2 g/kg/day. Our aggressive nutrition approach helps minimize the loss of protein and calories during the 6 hours without TPN.

Limitations of our study included broad categorization of amino acid profiles, thus limiting our ability to identify particular patterns of amino acid abnormalities in false positive results. Further research that examines the reduction in the false positive breakdown of amino acid levels from NBS in infants will be valuable to examine whether further methods are needed to reduce certain false positive amino acid profiles. Furthermore, our QI project was focused on practice in a single site and thus, studies that evaluate our protocol at different sites would be beneficial.

In conclusion, our results propose modifications to the NBS protocol that resulted in a significant reduction in the rate of false positives amongst all BW categories, with novel focus on infants with a BW < 1000 g. This easy-to-implement and safe protocol adds to the existing methods proposed to reduce false positive rates and delivers higher reduction than previously reported. Future research should examine the efficacy of this protocol in multicenter NICU settings to facilitate application to practice. Implementing a new NBS collection protocol also reduces the risk of negative consequences associated with false positive NBS results. This has the potential to decrease other factors including subsequent laboratory procedures, parenteral and infant stress, and reducing health care expenses.

Article Information

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Disclosures. The authors declare no conflicts or financial interest in any product or service mentioned in the manuscript, including grants, equipment, medications, employment, gifts, and honoraria. The authors had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.

Ethical Approval and Informed Consent. Given the nature of this study, the project was exempt from institution review board/ethics committee review and informed consent or patient assent was not obtained.

Submitted. July 19, 2020

Accepted. January 3, 2021

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References

1. American Academy of Pediatrics, Newborn Screening Authoring Committee. Newborn screening expands: recommendations for pediatricians and medical homes – implications for the system. *Pediatrics*. 2008;121(1):2007–3021.
2. Watson MS, Mann MY, Lloyd-Puryear MA, et al. Newborn screening: toward a uniform screening panel and system. *Genet Med*. 2006;8(suppl):251–252.
3. Zaffanello M, Zamboni G, Maffei C, Tatò L. Neonatal birth parameters of positive newborns at PKU screening as predictors of false-positive and positive results at recall-testing. *J Med Screen*. 2003;10(4):181–183.
4. Kanungo S, Patel DR, Neelakantan M, Riyali B. Newborn screening and changing face of inborn errors of metabolism in the United States. *Ann Transl Med*. 2018;6(24):468–473.
5. Slaughter JL, Meinzen-Derr J, Rose SR, et al. The effects of gestational age and birth weight on false-positive newborn-screening rates. *Pediatrics*. 2010;126(5):910–916.
6. Kaye CI, Accurso F, La Franchi S, et al; Committee on Genetics. Introduction to the newborn screening fact sheets. *Pediatrics*. 2006;118(3):1304–1312.
7. Lipstein E, Perrin J, Waisbren S, et al. Impact of false-positive newborn metabolic screening results on early health care utilization. *Genet Med*. 2009;11(10):716–721.
8. Gurian EA, Kinnamon DD, Henry JJ, Waisbren SE. Expanded newborn screening for biochemical disorders: the effect of a false-positive result. *Pediatrics*. 2006;117(6):1915–1921.
9. Schmidt J, Castellanos-Brown K, Childress S, et al. The impact of false-positive newborn screening results on families: a qualitative study. *Genet Med*. 2012;14(1):76–80.
10. Kelleher A, Clark R, Steinbach M, et al. The influence of amino-acid supplementation, gestational age and time on thyroxine levels in premature neonates. *J Perinatol*. 2008;28(4):270–274.
11. ten Hoedt AE, van Kempen AA, Boelen A, et al. High incidence of hypermethioninaemia in a single neonatal intensive care unit detected by a newly introduced neonatal screening programme. *J Inher Metab Dis*. 2007;30(6):978–983.
12. Hustace T, Fleisher JM, Sanchez Varela AM, et al. Increased prevalence of false-positive hemoglobinopathy newborn screening in premature infants. *Pediatr Blood Cancer*. 2011;57(6):1039–1043.
13. Morris M, Fischer K, Leydiker K, et al. Reduction in newborn screening metabolic false-positive results following a new collection protocol. *Genet Med*. 2014;16(6):477–483.
14. Blanco CL, Gong AK, Green BK, et al. Early changes in plasma amino acid concentrations during aggressive nutritional therapy in extremely low birth weight infants. *J Pediatr*. 2011;158(4):543–548.
15. Tim AT, Harmon HM, Nock ML, et al. Stopping parenteral nutrition for 3 hours reduces false positives in newborn screening. *Pediatric*. 2015;167(2):312–316.
16. Asghar A, Shabanova V, Mercurio MR, et al. A high rate of false positive newborn screening results in the neonatal intensive care unit. *J Child Adolesc Health*. 2019; 3(1):7–11.
17. Clark RH, Kelleher AS, Chace DH, et al. Gestational age and age at sampling influence metabolic profiles in premature infants. *Pediatrics*. 2014;134(1):e37–e46.
18. Chace DH, De Jesus VR, Lim TH, et al. Detection of TPN contamination of dried blood spots used in newborn and metabolic screening and its impact on quantitative measurement of amino acids. *Clin Chim Acta*. 2011;412(15–16):1385–1390.
19. Corkins MR, ed. *The A.S.P.E.N. Pediatric Nutrition Support Core Curriculum*, 2nd ed. Silver Spring, MD: ASPEN; 2015.