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Reduction in newborn screening metabolic false-positive results following a new collection protocol

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Purpose: Newborn screening includes testing for many metabolic diseases. False-positive results are higher among neonatal intensive care unit infants, resulting in increased confirmatory testing and family stress. Amino acid administration as a component of total parenteral nutrition is commonly used in the neonatal intensive care unit and suggested as a factor increasing false-positive results. The purpose of this study was to investigate the impact of a new sample collection protocol on false-positive results.

Methods: This was a 2-year retrospective cohort study. Infants were grouped by birth year into pre- and postprotocol implementation and stratified by birth weight category. In 2010, newborn screening samples were collected from all infants regardless of total parenteral nutrition administration. In 2011, the protocol was changed, and total parenteral nutrition was replaced with 10% dextrose in water (D10W) for 3 h before sample collection.

Results: Data from 539 neonatal intensive care unit admissions were reviewed. The new protocol reduced false-positive results for each birth weight group by at least 50% and overall by 74% ($P = 0.008$). The odds of having a false-positive result preintervention were 3.87 times higher than postintervention. The protocol reduced estimated costs by >80%.

Conclusion: A protocol interrupting total parenteral nutrition for 3 h before newborn screening collection resulted in a 74% reduction in false-positive results in a neonatal intensive care unit.

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Key Words: FPR; NBS; NICU; tandem mass spectrometry; TPN

INTRODUCTION

Inborn errors of metabolism are a group of rare, inherited disorders that, when left undiagnosed and untreated, can have devastating complications or even result in death. Infants with inborn metabolic diseases can be detected with biochemical testing even though they are often asymptomatic at birth. The goal of newborn screening (NBS) is to test all infants and properly identify those with a disorder early so that prompt therapy may be initiated. The American Academy of Pediatrics and the American College of Medical Genetics and Genomics recommend that all newborn infants undergo NBS shortly after birth.[1,2]

NBS testing began in the United States in the early 1960s with screening for phenylketonuria, and, over the years, it has evolved into a complex program testing for multiple conditions.[3,4] The number of diseases screened for has greatly increased with the availability of new technology like tandem mass spectrometry, which measures amino acids for the diagnosis of aminoacidopathies and acylcarnitines for the detection of fatty acid oxidation defects and organic adicemias using dried blood spots on filter paper.[4] The collection technique and sample requirements remain the same as those originally established; therefore, the use of tandem mass spectrometry is an efficient method to screen for many conditions without increasing the amount of blood required.[5] In 2005, the American Academy of Pediatrics endorsed the recommendations by the American College of Medical Genetics and Genomics to increase the number of disorders included in the newborn screen core panel to 29 identified conditions and 25 additional conditions that are part of the differential diagnosis in the core panel.[1,2]

Most infants are born healthy and discharged within 1–2 days after birth. Infants born ill or prematurely require longer hospitalization and often receive intensive medical care in a neonatal intensive care unit (NICU). Various medical therapies, including total parenteral nutrition (TPN), provided in the NICU and the liver immaturity of the preterm infant's metabolic system contribute to higher rates of presumptive positive NBS results in this group.[6–9] This population requires special planning and investigation into the best practice for collecting the NBS specimen in a manner to reduce the potential of a false-positive result (FPR), a result that is out of the range established by the NBS laboratory (presumptively positive), and additional testing confirms that no disorder exists. However, despite the importance of this problem, there is limited literature about how to decrease the FPR in the NICU population.

In 2009, the Clinical and Laboratory Standards Institute published the document entitled "Newborn Screening for Preterm, Low Birth Weight, and Sick Newborns".[10] This document

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suggests that if an infant is affected with a metabolic disorder, the NBS results will be grossly abnormal, and immediate follow-up is required.[10] Infants on TPN may have a NBS result with multiple minor amino acid abnormalities, and these patterns could be recognized by the NBS laboratory.[10] In these cases, follow-up is recommended when the infant is off of TPN.[10] There are no studies investigating how long the infant should be off of TPN for an appropriate specimen, and waiting until the infant is completely off of TPN may result in a delayed diagnosis.

In addition, although it may be possible for the NBS laboratory to estimate that a result is suggestive of TPN administration when several metabolites are marginally increased, it is not always possible to discern in a sick neonate if the increase in one metabolite is due to TPN administration, liver immaturity, or a real metabolic disease. This is particularly challenging as information about TPN administration at sample collection is not always provided to the NBS laboratory.

We present a study that was conducted to evaluate whether a change in the collection protocol for NBS specimens would reduce the FPR for metabolic disorders in a NICU.

MATERIALS AND METHODS

Participants

We conducted a retrospective cohort study in the Level IIIC NICU at Children's Hospital of Orange County (CHOC) using data from NBS results collected for 2 years: 1 January 2010 to 31 December 2011. As the study center was a children's hospital, all admissions to the study NICU were transported in from another facility. Only patients admitted from a single delivering hospital adjacent to the children's hospital, who had their initial NBS test collected in the study NICU, met study criteria and were included in the study. Two groups of infants meeting study criteria were compared. The preintervention group included all infants who were admitted to the NICU from 1 January 2010 to 31 December 2010 (n = 274). The second group included infants meeting study criteria in the year following a change in the protocol for NBS collection, 1 January 2011 to 31 December 2011 (n = 265). All data were collected retrospectively from review of the electronic health record. The study was approved by the Institutional Review Boards of CHOC and Brandman University.

Study variables

Information was retrieved from the participants' electronic health record by the investigators and included the following details: sex, birth weight, age in hours since birth at the time of specimen collection, TPN administration before or during specimen collection, and NBS test result. The primary independent variable studied was the administration of TPN before specimen collection to determine if interruption of this therapy in accordance with the new protocol reduced the number of FPRs. The dependent variable and main outcome measure was a report of a presumptively positive NBS test that was later confirmed as a FPR by additional testing. Additional variables such as sex, birth weight, and the infant's age in hours at the time of specimen collection were evaluated to compare the two groups. Supply utilization, personnel time, and the number of tests required to confirm a FPR were collected to complete a cost analysis.

Procedure

During 2010, NBS samples were collected from all infants regardless of TPN administration. In January 2011, the protocol was changed such that TPN was interrupted for 3 h before NBS sample collection for any infant receiving TPN. The period of 3 h without TPN was chosen as it mimics the usual fasting interval of a newborn. 10% dextrose in water (D10W) was infused at the same rate the TPN had been infusing during that time via a medication line. After the specimen was collected, the TPN was restarted. The standard intravenous system in the study NICU included trifuse tubing which allowed for infusion of TPN and medications or additional fluid, such as D10W, via a single site, so there was a minimal risk of infection from line access. As part of the revision of the NICU policy interrupting TPN, the policy was also revised to include a new recommendation from the California NBS Program that the specimen is ideally collected between 24 and 48 h of life. All specimens were collected by the NICU nursing staff via a single heel-stick sample on a filter paper supplied by the State Newborn Screening Program as recommended by the California Department of Public Health.[11]

For the purpose of the NBS test in California, amino acids and acylcarnitines are analyzed via tandem mass spectrometry technology.[11,12] California State Newborn Screening laboratories, like every state laboratory, determine the NBS collection protocol (single specimen) and the cutoff values for each analyte. Results that are out-of-range for the laboratory cutoff values are considered presumptively positive until additional testing confirms the presence or absence of a disorder.

Data analysis

All data were collected from the study participants' electronic health record, coded, and stored using a password-protected personal computer. Data were then entered and analyzed using the software package SPSS 19.0 for Windows (SPSS, Chicago, IL). Statistical significance was set at a P value of 0.05. The goal was to compare the differences in the FPR rates between groups using a χ^2 test of homogeneity. Odds ratios with a 95% confidence interval were reported for significant χ^2 values. A two-tailed independent groups t-test with 95% confidence interval for the effect size was used to compare the two groups in mean birth weight and mean hour of life at time of the NBS sample collection. For subgroup analysis, when the assumptions of a χ^2 test were not met due to small sample size, the two-sided Fisher's exact test was used.

Exact costs for supplies and confirmatory tests were calculated for a cost analysis. Estimates of personnel time and salaries were based on telephone or personal interviews from the health-care professionals directly involved with the NBS program at the study hospital.

RESULTS

A total of 539 newborns were screened in the 2-year study period, 274 in the preintervention period and 265 in the year following the change in NBS collection protocol (Table 1). The two groups were not statistically different in sex ($x_1^2 = 0.49$; $P = 0.49$) or birth weight category ($x_2^2 = 0.17$; $P = 0.92$; **Table 1**). As shown in Table 2, the mean birth weight between the two groups was not statistically different ($t_{534} = 0.04$; $P = 0.97$). The mean time of specimen collection was different between groups, preintervention mean = 51.3 h and postintervention mean = 37.6 h ($t_{502} = 5.89$; $P < 0.001$; Table 2).

There was a significant reduction in the number of FPRs from 16 (5.8%) in the preintervention group to 4 (1.5%) in the postintervention group (Table 3). The odds of having a FPR in 2010 were 3.87 times higher than that in 2011 (Table 3).

The participants were evaluated as to whether they were receiving TPN administration at the time of specimen collection. During the preintervention period, 169 patients (62%) were on TPN at the time of NBS specimen collection. Among these 169 patients, 16 patients (9.5%) had a FPR, whereas none of the 105 patients not on TPN had a FPR ($x_1^2 = 10.56$; $P = 0.001$).

Analysis by birth weight category showed that the new protocol reduced the FPR by at least half in each of the three groups. The reduction was greatest in the infants with a birth weight >1,501 g (Table 3).

Analysis of the most frequent causes of FPR revealed that amino acid abnormalities were more frequent than acylcarnitine abnormalities (Table 4). Many patients had more than one marker above the cutoff range. The most common amino acid abnormalities found were an increase in methionine and/or leucine. Notably, the increases in the above analytes were the only abnormalities found in the 2011 cohort.

The costs of supplies and confirmatory testing during the preintervention period for the 16 FPRs totaled US$4,699, whereas the costs in the postintervention period for 4 FPRs were US$911. There was an overall cost avoidance of US$3,788 or 81% (Table 5). In addition, personnel time was estimated between 13 and 14 h per FPR (NICU nursing: 1 h; NICU physician: 1 h; NBS program coordinators: 5 h; metabolic genetic counselors: 5–6 h; and metabolic physicians: 0.5–1 h). The personnel cost for the entire team was estimated to be US$713 per FPR or $11,400 for all the FPRs in the preintervention period. The postintervention estimation of personnel cost for 4 FPRs was US$4,015, which included additional time for NICU nursing to interrupt the TPN infusion. This represents a savings of 65% in personnel costs compared with the preintervention period.

Table 1 Sex and birth weight group

	Preintervention, *n* (%)	Postintervention, *n* (%)	χ^2	df	*P*
Sex			0.49	1	0.49
Male	155 (56.6)	142 (53.6)			
Female	119 (43.3)	123 (46.4)			
Birth weight group			0.17	2	0.92
<1,000 g	20 (7.3)	17 (6.4)			
1,001–1,500 g	25 (9.1)	25 (9.4)			
>1,501 g	229 (83.6)	223 (84.2)			

DISCUSSION

The odds of an infant in the NICU having a FPR on the NBS test were nearly four times higher in the year before implementing a protocol to interrupt TPN administration for 3 h before

Table 2 Mean birth weight and time of newborn screening specimen collection

	Preintervention	Postintervention				
	Mean (SD)	Mean (SD)	*t*	df	*P*	95% CI
Birth weight	2,429.54 (907.04)	2,426.73 (946.25)	0.035	534	0.97	−153 to 160
Age (hours since birth) for specimen collection	51.32 (30.88)	37.62 (22.68)	5.89	502	<0.001	9.11 to 18.3
Range (h)	4–329	8–341				

Table 3 Newborn screening test results total and by birth weight category

	Preintervention (*N* = 274)		Postintervention (*N* = 265)	
	Negative, *n* (%)	False-positive, *n* (%)	Negative, *n* (%)	False-positive, *n* (%)
Birth weight				
<1,000 g	13 (65.0)	7 (35.0)	14 (82.4)	3 (17.6)[a]
1,001–1,500 g	23 (92.0)	2 (8.00)	24 (96.0)	1 (4.00)[b]
>1,501 g	222 (96.9)	7 (3.06)	223 (100)	0 (0.00)[c]
Total	258 (94.2)	16 (5.83)	261 (98.5)	4 (1.50)[d]

CI, confidence interval; OR, odds ratio.

[a] $x_1^2 = 1.4$; $P = 0.29$ (Fisher's exact test); OR = 1.98; 95% CI: 0.61–6.5. [b] $x_1^2 = 0.4$; $P = 1.0$ (Fisher's exact test); OR = 2; 95% CI: 0.19–20.7. [c] $x_1^2 = 6.9$; $P = 0.02$ (Fisher's exact test). [d] $x_1^2 = 7.1$; $P = 0.008$; OR = 3.87; 95% CI: 1.31–11.42.

Table 4 False-positive results by analyte

	Preintervention (*n* = 16)			Postintervention (*n* = 4)		
	<1,000 g (*n* = 7)	1,001–1,500 g (*n* = 2)	>1,501 g (*n* = 7)	<1,000 g (*n* = 3)	1,001–1,500 g (*n* = 1)	>1,501 g (*n* = 0)
Elevated AA		*n* (%)			*n* (%)	
Methionine	4 (57)	2 (100)	7 (100)	3 (100)	1 (100)	–
Leucine[a]	7 (100)	1 (50)	7 (100)	–	–	–
Leucine/alanine	–	–	3 (43)	–	–	–
Phenylalanine	1 (14)	–	3 (43)	–	–	–
Phenylalanine/ tyrosine	1 (14)	–	–	–	–	–
Tyrosine	–	–	–	1 (33)	–	–
Citrulline	1 (14)	–	–	–	–	–
Ornithine	1 (14)	–	–	–	–	–
Total number of AA elevations	16	3	20	4	1	0
Elevated AC		*n* (%)			*n* (%)	
C3/C2 ratio	–	–	1 (14)	–	–	–
C3DC	–	–	1 (14)	–	–	–
C4	1 (14)	–	–	–	–	–
C5	4 (57)	–	–	–	–	–
Total number of AC elevations	5	0	2	0	0	0

AA, amino acid; AC, acylcarnitine; MS/MS, tandem mass spectrometry; NBS, newborn screening.

[a]Leucine, isoleucine, alloisoleucine, and hydroxyproline are isobaric amino acids, and most NBS laboratories using MS/MS technology do not differentiate among them in the initial screening test.

Table 5 Cost analysis

Item	Preintervention	Postintervention
Confirmatory testing[a]	US$4,653	US$434
Supplies for testing[b]	US$46	US$12
Supplies for new protocol[c]	US$0	US$465
Total	US$4,699	US$911

FPR, false-positive result.

Information is based on 16 FPRs in preintervention and 4 FPRs postintervention years.

[a]Each FPR required multiple confirmatory tests that ranged in cost from US$8.82 to US$210.63. [b]Supplies include lancet and heel warmer for blood draws and urine bag for urine tests. [c]New protocol supply costs are US$3.00 per patient (bag of D10W, intravenous spike, and syringe), 155 patients in postintervention year.

specimen collection. The protocol was evidence based, safe, cost effective, and resulted in no adverse effects.

The State of California Newborn Screening Program mandates that any diagnosis of a metabolic disorder included in the NBS panel be reported to the State, regardless of the age at diagnosis. This surveillance process assures that any false-negative result will be identified. The Metabolic Program at CHOC would have been the referral center for any child in our study population suspected to have a metabolic disorder. At the submission of this manuscript, the patients born in the postintervention period would be 18–30 months old, an age range during which symptoms related to a metabolic disease would likely be apparent. As of the date of submission of this manuscript, no false-negative results have been identified in this population.

TPN is a fundamental component in the nutritional management of the preterm infant and a common therapy in the NICU. It provides nutrients, minerals, and vitamins for growth and development. Early initiation of TPN has become the standard of care for the preterm infants to prevent nutritional deficits, which could result in growth restriction.[13] It is known that poor growth during early hospitalization can lead to abnormal neurodevelopmental outcomes.[14] The preterm infant is born without the benefit of fetal protein accretion that would have occurred in the last trimester of gestation, and protein administration as an amino acid solution is well tolerated, improves growth, and improves neurodevelopmental outocmes.[14–17]

It has been found by many authors that the infusion of amino acids increased NBS FPR.[6,8,9,18] However, as TPN is essential to optimize nutrition, simply delaying the start of TPN administration until after the NBS specimen collection is not recommended. Our protocol achieved a reduction in the FPR from 5.8 to 1.5%, while maintaining the benefits of early nutritional support and avoiding the side effect of an increased FPR.

The detailed components of the TPN composition during the study were not collected. The standard amount of amino acid given to the infant receiving "starter TPN" in the study NICU is 2.8 g/kg/day. Amino acid administration may be increased up to a maximum of 3.5 g/kg/day after 48 h of life. Therefore, as the majority of specimens were collected before 48 h of life,

the infants would have received amino acid administration of 2.8–3 g/kg/day, and this was similar in both the pre- and postintervention groups.

The FPR of 5.8% found in all patients in the study Level IIIC NICU was higher than what has been reported for the overall newborn population, where rates range from 0.11 to 0.3%.[5,6,12,19] Our results do, however, agree with others who have demonstrated a higher FPR in premature infants or in those admitted to a NICU.[6–9,20] The results also indicate that an increased FPR can be expected with a lower birth weight as has been shown by others.[4,7–9,20]

The highest FPR was seen in infants with a birth weight <1,000 g when compared with the other birth weight groups. This is likely related to hepatic immaturity, which delays the detoxification ability of the liver.[21] Nevertheless, our protocol achieved a reduction in the FPRs from 35 to 17.6% in this birth weight category (**Table 3**). Developing different laboratory cutoff values for term versus preterm infants or for infants receiving TPN may be a strategy to further decrease FPRs in this population.

It has been well established that preterm infants have higher rates of FPRs, and it is also known that infants in a NICU also have increased rates of true disorders.[6–9] In an effort to minimize possibly missing a true disorder for all conditions included in the NBS program, some programs have implemented guidelines that recommend obtaining a second or third sample at different ages.[10,22] The study protocol we investigated, which involved interrupting TPN and reducing FPRs, could also be incorporated into a multiple specimen collection program avoiding parenteral anxiety and the potential risk of delaying the diagnosis of a metabolic disorder that could result from waiting until the patient is completely off TPN to collect an additional sample.

The most common amino acid abnormalities in our study were increased methionine and leucine. Both abnormalities appeared frequently in the same patient. Many NBS laboratories use different amino acid ratios to increase the sensitivity and specificity of the NBS results for disease detection.[23] California uses leucine values and the leucine to alanine ratio as markers for maple syrup urine disease.[12] As shown in **Table 4**, in the preintervention group, leucine levels were increased in 15 patients, whereas the leucine/alanine ratio was elevated only in 4 patients. Therefore, only four of these patients were identified as presumptively positive for maple syrup urine disease by the California Newborn Screening Program.

Methionine levels were elevated in 13 of the 16 FPRs in the preintervention period and in all 4 FPRs in the postintervention period. All of these patients were identified as presumptively positive for hypermethioninemia. Some NBS programs use a methionine to phenylalanine ratio to improve sensitivity and specificity for the diagnosis of hypermethioninemia. This ratio is not currently used in California. To assess the potential impact of adding this marker, we calculated the methionine/phenylalanine ratios for all patients with a FPR and used a hypothetical cutoff value of 1, based on California normal population distribution.[24] Even by combining both methioinine levels and the methionine/phenylalanine ratios, 10 of the 13 patients with an elevated methionine level in the preintervention period and all 4 patients in the postintervention period would still have been identified as presumptively positive for hypermethioninemia (data not shown). This demonstrates that although the use of a ratio has value to decrease the FPRs for certain disorders, it does not decrease the risk of a FPR for hypermethioninemia. It is likely that this amino acid is increased out of proportion to the other amino acids due to the preterm liver immaturity and TPN administration; therefore, interrupting TPN for a short time as proposed in our protocol has a significant impact in this population. Periodically, the reference ranges of the analytes measured in NBS programs are adjusted, and ratios are added to improve the specificity of the testing. In August 2010, the California NBS Program added various ratios and adjusted several reference ranges. The two changes relevant to our patient population included adjusting the cutoff for methionine from 0–75 to >5 to <100 and adding the C3/C5 ratio. The change in methionine would have decreased the number of cases flagged for that analyte from 13 to 12 in the preintervention period. However, the patient in question still had elevations in leucine and in leucine/alanine ratio. The addition of the C5/C3 ratio would have reduced the cases flagged for isovaleric acidemia; however, these four cases also had elevations in additional analytes. In summary, even if we applied the ratios and reference range changes implemented in August 2010, to all of our cases, it would not have affected the number of children that required confirmatory testing.

We also analyzed our data using the Region 4 Stork (R4S) postanalytical tools to determine how likely it was for each of our cases to be flagged due to the influence of TPN rather than due to a true metabolic condition.[23–25]

After entering results for several analytes, the tool generates a score that predicts the likelihood that the: (i) condition is very likely due to TPN, (ii) condition is likely due to TPN, (iii) condition is possibly due to TPN, and (iv) profile is not informative for TPN.[23–25] Our analysis revealed that 81.25% of the preintervention and 100% of the postintervention cases were categorized as likely or very likely to be due to TPN. Therefore, the R4S one condition tool supported our contention that TPN is a major factor influencing the FPRs of NBS cases. Use of the tool at the screening laboratory level upstream of the NICU or metabolic center setting might provide an excellent method of reducing the number of FPRs due to TPN. This approach should be validated by each state's NBS laboratories. Our protocol of interrupting TPN would reduce the number of FPRs that would require the use of the R4S tool to begin with. Therefore, both strategies may be beneficial in decreasing the number of FPRs and are not mutually exclusive. As our intervention reduced the number of FPRs from 16 to 4, it is possible that the proportional difference in the R4S scores between the pre- and postintervention periods are related to the lower number of FPRs.

Many times, intralipids are administered with TPN, and in our study, 36.5% of patients in the preintervention period and

1.5% in the postintervention period were receiving intralipids at the time of specimen collection. However, only one patient in the preintervention period flagged positive for a fatty acid oxidation defect; therefore, it appears that intralipids do not play a major role in the FPRs in the NICU population.

Although we acknowledge that the mean collection time between the two groups was different, 93.4% of infants in the preintervention group and 97.3% of the infants in the postintervention group had the NBS specimen obtained within the time frame recommended by the State of California Newborn Screening Program, and a majority (54.8% in the preintervention year and 91.7% in the postintervention year) had the specimen collected between 24 and 48 h of life. The time difference could be considered a limitation of this study; however, this is an evidence-based protocol and reflects a complete capture of all patients meeting criteria and a true clinical application in a NICU where there are varied reasons for outliers in the NBS collection times.

Other studies have shown that specimen contamination by TPN solution can lead to a FPR.[18] It is important to clarify that all of the NBS samples in our study were collected via heel-stick and the FPRs were not related to contamination of a specimen by TPN in the intravenous line.

The results of this study demonstrate that the number of FPRs can be reduced in all birth weight categories. However, because the number of FPRs was reduced significantly in the postintervention period, despite a large overall population, the sample size of infants with a FPR was not large enough to statistically analyze all variables. Data collection continues as other NICUs in the region implement the new specimen collection protocol.

NBS is a costly mass-screening program that provides important health benefits and compares favorably with other preventative and screening programs such as breast and prostate cancer.[26,27] However, NBS becomes more costly with the additional confirmatory testing required for FPRs. The new protocol yielded a reduction in over 80% of the costs associated with the confirmatory testing for FPRs in the study NICU (**Table 5**). As NBS is an interdisciplinary process, a decrease in FPRs reduces time utilization for multiple health-care providers from the NICU (physicians and nursing), laboratory, state NBS programs, and metabolic specialists (physicians and genetic counselors). The cost analysis for personnel time was determined based on interviews of the interdisciplinary team. Intangible benefits, such as reduced unnecessary laboratory draws for the fragile NICU infant and decreased parental stress and anxiety as has been shown to occur in parents of infants with a FPR, are also achieved.[28]

CONCLUSION

This study showed that implementation of an evidence-based practice, replacing TPN with a D10W infusion for 3 h before collecting the NBS specimen, reduced false-positive NBS results in a Level IIIC NICU. This simple protocol improved care by reducing unnecessary laboratory draws, pain, and stress for the infants and their parents and reduced expenses for the health-care system. Further investigation with a larger sample size should be considered.

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DISCLOSURE

The authors declare no conflict of interest.

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